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Mechanism of Microbiological Contamination of Jet Fuel and Development of Techniques for Detection of Microbiological Contamination

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FOREWORD

This is the fourth quarterly progress report prepared under Contract AF 33(657)-9186, "Mechanism of Microbiological Contamination of Jet Fuels and Development of Techniques for Detection of Microbiological Contamination." This contract was initiated by the Air Force Aero Propulsion Laboratory, Research and Technology Division, Wright-Patterson Air Force Base. Mr. Jack Fultz is the Project Engineer.

This report concerns work done from 1 February 1964 to 1 June 1964.

ABSTRACT

Progress was made during this period in understanding and partially solving the problem of jet fuel contaminants, including microorganisms, surface-active materials, iron rust, water, and extraneous materials as they pertain to fuel properties, sludge formation, and their degree of participation in the corrosion and degradation of aircraft materials of construction throughout the fuel system. Several major developments in this area of research were accomplished. The first was the establishment of concepts that explain how various growth media cause the corrosion of aluminum. A study of the corrosion in simulated water bottoms revealed that ions, such as iron and calcium, cause aluminum corrosion like that caused by sodium chloride. However, other ions in the media, such as nitrate and phosphate, prevented aluminum corrosion. The mechanism of microbial corrosion appears to depend on the removal of these corrosion inhibitors by microbial growth. Aluminum corrosion was also caused by another mechanism as revealed by the corrosion produced by the growth of fuel isolates in media containing protein, peptides, and amino acids. This type of corrosion was not inhibited by nitrate. The second development was in understanding the metabolic mechanisms which operate in the production of fuel contaminants such as emulsions and sludges. Compounds produced by microorganisms grown in fuel were isolated and partially characterized. One potentially effective method of controlling the microorganisms in fuel was discovered. The respiration and viability of these isolates were especially sensitive to inhibition by short-chain unsaturated hydrocarbons. The third development was in correlating the breakdown of coatings on aluminum with microbial growth. Microorganisms utilized these coating materials as sources of nitrogen and carbon for growth. The fourth development was the application of several different detector systems to the study of fuel contamination and corrosion.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF ILLUSTRATIONS (Cont.)

LIST OF ILLUSTRATIONS (Cont.)

LIST OF ILLUSTRATIONS (Cont.)

LIST OF TABLES

I. INTRODUCTION

The fact that microorganisms grow in hydrocarbon substrates, especially fuels, has been known for many years. The organisms are associated with the corrosion of iron, the pollution of water, the decomposition of gasoline additives, the formation of microbial masses at water-oil interphase, the clogging of fuel lines, the degradation of sealants and topcoats, and the corrosion of aluminum in the wing fuel tanks of jet aircraft. The manner of the direct involvement of microbes and the mechanisms by which these various difficulties are brought about by microbial agents remains poorly understood. It is felt that an understanding of these mechanisms can be gained from the studies conducted in this program.

A mechanism study has been designed to determine how each of the fuel contaminants (microorganisms, water, surface-active materials, and iron rust) causes alteration (corrosion, sludge formation, degradation of fuels, sealants, and topcoats, etc.) in fuel systems. Research on mechanism was principally concerned with defining the role of microorganisms in fuel contamination.

During the fourth quarter of the contract, definite relationships between microbial growth and fuel contamination have been established and some of the mechanisms by which microorganisms cause corrosion of aluminum alloys have been classified.

Research was performed also on the conditions of environment which bring about aluminum corrosion by microorganisms. The hypothesis was proposed and tested that microbial growth results in the removal of naturally occurring inhibitors from fuel-water systems and leaves unused corrosive concentrations of elements such as iron, calcium, and chlorine.

The alternate hypothesis that organisms produce corrosive compounds was tested, and this hypothesis appeared to be partially proven by the ability of cells to cause corrosion when grown in certain media.

Studies on the intermediary metabolism and respiratory mechanism of hydrocarbon-oxidizing organisms were continued. The results of these studies appear to justify the belief that a large measure of control can be exercised over the production of microbial fuel contaminants by strictly chemical means.

A method was developed for detecting the tendency of a medium to corrode aluminum. This detection device should have important implications both for the detection of fuel contaminants, such as rust and heavy metals, and for detection of biological contaminants, such as microorganisms or their possible corrosive metabolic products.

II. SUMMARY AND CONCLUSIONS

During the past four quarters the contamination of jet fuel systems and the corrosion of aluminum alloys by microorganisms has been investigated at the cellular level. The study has been concerned with the mechanisms by which microorganisms cause the corrosion of aluminum alloys and the mechanism by which microbial growth contributes to the problem of fuel contamination.

During the third and fourth quarters the effects of growth media constituents on the occurrence of aluminum corrosion received attention, and effort was devoted to the design of microbial growth media which of themselves neither cause corrosion nor inhibit the corrosion caused by microorganisms. The design of such a medium was a formidable task, but it was one that was essentially solved by research done during the fourth quarter of this contract.

The relationship of the relative concentrations of mineral contaminants to aluminum corrosion was defined, and the ability of microorganisms to alter this ratio and increase the corrosivity of media was demonstrated.

In the third quarter attempts to study hydrogenase production by fuel isolates led to the discovery that nitrate ion inhibits aluminum corrosion and also exercises a known inhibitory influence on the synthesis of this enzyme system. These observations led to the speculation, since proven valid, that microorganisms may cause corrosion of aluminum by the removal of certain mineral corrosion inhibitors.

The concentration of nitrate and phosphate which prevents the corrosion of aluminum by biologically essential ions at concentrations used in growth media was ascertained. The ability of fuel isolates to grow on media with nitrate as the only nitrogen source was established. Growth on such media necessitated the removal of the corrosion inhibitors, nitrate, and phosphate. Inoculated media low in nitrate caused aluminum corrosion more rapidly than media high in nitrate concentration or in uninoculated controls. A similar relationship was evident in media containing various concentrations of phosphate.

During the early part of this study, media high in nitrate concentration with high concentrations of cells were not observed to corrode aluminum, but this medium contained ammonium ion in addition to nitrate ion. Studies have since shown the ability of ammonia to spare nitrate and prevent the utilization of this corrosion inhibitor by microorganisms.

The physiological activity of fuel isolates was such that the ammonium ion was preferentially used in media with both ammonia and nitrate as nitrogen sources. In media, such as Bushnell-Haas medium, the hydrogen ion concentration increases rapidly with respect to growth and appears to limit the yield of organisms. Hydrogen ion did not increase appreciably with nitrate

as a nitrogen source. Such increases did not take place even when media were prepared with low buffering capacity from phosphate. The ability of ammonia to inhibit the utilization of nitrate, a corrosion inhibitor, emphasized the large number of biologically important variables that control the ability of organisms to cause corrosion. It was concluded that a simple relationship between nitrate concentration and corrosion inhibition appeared to prevail in media without microorganisms, but the relationship became more complex in media with microorganisms containing high concentrations of nitrate. A corrosion mechanism that did not appear to depend on microbial depletion of nitrate was evident at high concentrations of this anion.

Microbial corrosion occurred in media with high-nitrate ion concentration in the absence of other nitrogen sources, but long periods of time were required to obtain this effect. In such media fuel-extractable compound(s) were produced, and submerged aluminum coupons were coated with deposits of organic material. In these media the fuel isolates with fuel as the only source of carbon attained populations of 10^{10} cells per ml, but unlike cells grown on lower nitrate concentration they did not tend to rise into the fuel layer.

J. Takahashi et al. (1) and K. Yamada et al. (2) report that microorganisms excrete protein and protein-like material into growth media during hydrocarbon oxidation. It may be supposed that water bottoms also contain organic contaminants and an investigation of a corrosive mechanism in which these reactants participate was begun with products of microbial metabolism such as proteins, peptides, and amino acids. These products were shown to corrode aluminum over long periods of time, but when microorganisms isolated from fuel were inoculated into such media with a jet fuel overlay, aluminum corrosion took place in large areas in a short time. The corrosion produced by microorganisms in these media containing proteinaceous materials was not inhibited by nitrate, and it appears to be different in character and represents the action of an important mechanism which differs from that operative in strictly mineral media with hydrocarbon overlays. Mixed cultures from Ramey have been used, but not actual water bottom. Corrosion by a water bottom obtained from a storage tank of Hotchkiss Oil Co., Fredericksburg, Va. was inhibited, however, by nitrate, and it may have resulted from a depletion of natural inhibition by microbial growth.

Two electrochemical techniques were employed to study the corrosive process. A polarographic technique was used to determine the corrosion products formed on aluminum and the pulse polarization technique was employed to confirm the importance of the minerals in causing and preventing corrosion of aluminum.

Classical polarographic techniques were used in an effort to follow the time course of aluminum alloy corrosion. These measurements suggested that the course of aluminum alloy corrosion was accompanied by the liberation of other ions of the alloy. This observation implied the operation of an autocatalytic process in which liberated ions such as copper catalyzed the further corrosion of aluminum and the further liberation of the ion catalyst.

A method was developed for detecting the tendency of a medium to corrode aluminum. The method depended on pulse polarization activation analysis. Aluminum corrosion was indicated long before it became visibly evident. The method also showed the ability of compounds to inhibit aluminum corrosion.

A study was started on the mechanism of hydrocarbon oxidation by fuel organisms because it was believed that this study would furnish clues to the mechanism of emulsion formation and corrosion. Fuel isolates were found which were constitutively adapted to the oxidation of jet fuel hydrocarbons while others required a period of adaptation to hydrocarbon oxidation. Fuel isolates oxidized jet fuel almost as rapidly as glucose and were variable in their requirements for minerals to effect their fuel oxidation. Acid production occurred with fuel oxidation. Tests were made of the possibility of controlling the growth of fuel organisms by using respiratory inhibitors but the respiration of fuel isolates was surprisingly resistant to azide and 2,4-dinitrophenol inhibition. But respiration of fuel isolates was stopped by 2-hexene or 1-heptene alone or when mixed with either glucose or fuel. In addition to preventing substrate oxidation, these unsaturated compounds were found to be biocidal. This suggested the possibility of designing water-soluble compounds with unsaturated hydrocarbon moieties for the control and study of metabolic fuel contamination processes.

Jet fuel is a protein denaturant and part of the essential structure of jet fuel organisms is made of protein. An effort was made to determine how fuel organisms, which contain protein, maintain their integrity in the presence of this denaturant. Organisms which could not utilize hydrocarbons as a carbon source were killed by exposure to JP-4 fuel, whereas fuel isolates survived.

In general, the fuel isolates studied were grown in Bushnell-Haas medium with a jet fuel overlay and the cell mass remained homogeneously distributed in the aqueous phase. But some fuel isolates were obtained which rose to the top of the aqueous phase and penetrated the fuel overlay. These organisms appear to be potential fuel layer contaminants. The specific gravity of these cells was such that they were concentrated at the top of the centrifuge tube when subjected to centrifugation. These cells were metabolically active and appeared to be similar to other fuel isolates. The occurrence of this phenomenon depended on the age of the cells and the nitrate concentration of the medium. Attempts were made to account for the tendency of some fuel-grown organisms to enter the fuel phase and to acquire a specific gravity less than water. Analysis revealed that such cells contained a large proportion of lipid material in the form of both fatty acids and esters of fatty acids. It was concluded that fuel isolates do produce substances which may carry both cells and contaminating debris into the fuel phase of a fuel-water system.

Another compound produced by microorganisms which entered the jet fuel phase was analyzed. This was a yellow compound extracted by jet fuel during microbial growth at high nitrate concentration. This compound was extracted

into water at pH 11. The compound is therefore protonated at the hydrogen ion concentration of the growth medium. A spectrophotometric titration indicated a pK of about 10.8. Absorption characteristics of the compound were recorded.

Bacteria used in this study were shown to be capable of using coatings as the sole source of carbon and nitrogen for growth. The ease with which different types of coatings were metabolized varied with the type of coating.

An example of coating failure correlated with microbial growth has been described where the coating blistered only when microbial growth was present.

Preliminary experiments indicate that coatings were used more readily as a carbon than as a nitrogen source, based on the total cell numbers produced.

Other data suggest that some coatings contain inhibitory substances that may or may not be removed by hot water extraction. Chromate-polymerized sealants exercise an inhibitory effect on growth whether extracted or not.

III. FUTURE WORK PLANNED

A. Work for the next quarter will include a study of the metabolism of hydrocarbons and environmental conditions that influence the production of emulsions and sludges by microbial fuel contaminants. The particular effect of 2-hexene, 1-heptene, and long-chain unsaturated hydrocarbons on mechanisms of microbial contamination will be investigated. Attempts will be made to understand the deleterious effects of these unsaturated hydrocarbons on respiration and survival in terms of the singular physiology of organisms isolated from fuel.

The relationship of fuel contaminants, such as rust and other minerals, to emulsion and sludge formation will be studied. The ability of fuel isolates to synthesize these contaminants in the presence of various metabolic inhibitors will be studied with the objective of elucidating metabolic pathways important to fuel contamination production.

B. A study will be made of the biochemical mechanisms by which fuel microorganisms transform fuel hydrocarbons into acids, esters, and contaminants which are soluble in fuel. Attempts will be made to characterize the active sites of enzymes that function in the production of emulsions and acids from jet fuel. The susceptibility of these enzymes to chemical control will be tested, and the effect of cell-free extracts and purified enzymes from fuel isolates on aluminum corrosion will be explored. A study will be made to determine whether or not the oxidation of hydrocarbon by microorganisms is enzymically coupled with the reduction of medium components such as carbonate, nitrate, phosphate, etc. The oxidation of unsaturated hydrocarbon biocides also will be studied.

C. The metabolic products which contaminate fuel, such as surface-active agents, will be characterized with respect to chemical composition, ability to react with aluminum alloys, and ability to react with sealants and coatings. The material which makes some fuel microorganisms float on water surfaces and penetrate the fuel phase of a fuel water system will be extracted and analyzed. The conditions causing the phenomenon will be studied. The occurrence of this alteration in specific gravity of a number of fuel isolates will be sought along with its cause in terms of specific chemical entities, and the mode of biosynthesis. The cell wall material of fuel isolates grown on fuel and on carbohydrates will be compared with the cell walls of non-fuel oxidizing organisms.

The separation of lipid material produced by fuel isolates oxidizing jet fuel will be analyzed by silicic acid chromatography, and the purified products obtained will be analyzed further for ester content and reactivity with fuel system components.

D. During the next quarter, work on microorganisms attacking coatings will continue. The emphasis will shift to specific organisms that have adapted to the substrates discussed in this report. Fungi isolated from

soil burial tests with coatings will be used for coating deterioration as well as other cultures from fuel tanks.

Tests on coated coupons will continue and better techniques for resistance measurements will be explored to determine whether or not a technique for evaluating failure of coatings can be developed.

Tests using soil burial as an enrichment technique are continuing as are other isolations of fungi and bacteria adapted to the utilization of coatings.

Nitrogen balance determinations will be made to determine whether microorganisms actively remove nitrogen from coatings.

The pulse polarization method will be applied to the analysis of the effect of inhibitors on aluminum in a large number of organic and mineral media. Attempts will be made to follow the enzymatic utilization of corrosion inhibitors by changes in the pulse polarization patterns.

IV. EXPERIMENTAL WORK

A. Mechanisms of Aluminum Corrosion

The present study concerns the particular properties and activities of microorganims which are believed to be related to the contamination of jet fuel and the corrosion of aluminum alloys.

Work was begun on the testing of the four possibilites by which microorganisms might cause corrosion of aluminum:

(1) Microbial growth and metabolism cause changes in the proportions of inorganic chemical compounds in growth media and by this means they diminish the quantities of corrosion inhibitors present and thus cause corrosion.

(2) Microbial metabolic products act as mediators in the corrosive process by stabilizing the oxidation-reduction potential, or by complexing metal ions and thereby shifting the chemical equilibrium in favor of corrosion.

(3) Microbial metabolism effects changes in the electrochemical properties of very confined areas such as microcolonies between metal and topcoat surfaces which establish centers of galvanic activity and thus cause corrosion.

(4) Microorganisms directly affect metal surfaces and cause the transfer of electrons from the metal to some physiologically produced electron receptor. This would be an activity analogous to that believed to occur with Desulfovibrio.

The probability exists that no one mechanism alone causes corrosion but, rather, a combination of many mechanisms which change in importance as conditions are varied. Present studies are concerned especially with testing the first and second hypotheses.

1. Corrosion Caused by Bushnell-Hass Medium Components

The first studies of microbial corrosion of aluminum carried out were concerned with a duplication of the work of others. These studies indicated that a casual relationship between microbial growth and aluminum corrosion could not be unequivocally established. Studies first referred to in the third quaterly report showed that the elimination of nitrate from a medium containing iron, calcium, magnesium, phosphate, ammonia, and sulfate caused the medium to become corrosive to aluminum alloys. This observation revealed that media often used for the growth of hydrocarbon oxidizing organisms, like Bushnell-Haas medium, would have prevented the action of corrosive compounds should they have been produced by microorganisms as well as inhibit the corrosive activity of ions frequently present in media supporting microbial growth.

To evaluate the contribution of microbial growth to aluminum corrosion, it was first necessary to determine the ability of the individual ions of the growth medium to cause corrosion in the absence of inhibitors such as nitrate and in the absence of microorganisms.

A preliminary screening of growth medium ions for ability to cause corrosion showed that only calcium chloride and ferric sulfate resulted in extensive corrosion of the alloys 2024 and 7075 within short time periods. Corrosion by magnesium chloride was variable and in general did not occur.

Tests for aluminum corrosion were made by submerging coupons of the alloys in the test solution with a jet fuel overlay and placing the system on a rotary shaker at 30°C for variable lengths of time. Following this treatment, coupons were removed, rinsed carefully in distilled water, blotted dry, and photographed. The pictures in Figure 1 show the effect of different $CaCl_2$ concentrations on the corrosion of aluminum alloys. Calcium sulfate produces a similar type of aluminum corrosion while the sulfate ion itself appeared to very mildly passivate the corrosion of aluminum. Therefore, the corrosion observed has been attributed primarily to the action of the cation. The data in Figure 1 show that 10^{-4} M $CaCl_2$, which is used in Bushness-Haas (BH) medium, is sufficient to cause the corrosion of aluminum alloys.

In microbial growth media at pH 7, the ferric ion present is hydrated and it is present in amounts that exceed the solubility of ferric hydroxide. The corrosion of aluminum alloys by solutions with different quantities of rust, $Fe(OH)_3$*, is shown in Figure 2. It was apparent that ferric ion, as used in BH media (3×10^{-4}M), was decidedly corrosive to the two alloys tested.

Preliminary studies indicated that both phosphate and nitrate prevented the corrosion of aluminum caused by corrosive ions. Further studies were performed to establish the concentration levels of these inhibitors which were effective against various concentrations of substances which appear to stimulate aluminum corrosion.

Tests were set up to determine the concentration of nitrate which would prevent the corrosion of aluminum in sterile solution caused by biologically essential ions at concentrations used in the BH growth medium.

Figure 3 shows the inhibition of corrosion caused by 8×10^{-4}M $Fe(OH)_3$ at various concentrations of nitrate. The half-maximum corrosion inhibition for the time interval observed was at 5.9×10^{-4} M KNO_3.

The concentrations of nitrate inhibiting 50% aluminum corrosion caused by 8×10^{-4}M $CaCl_2$ and NaCl were determined respectively from Figures 4 and 5.

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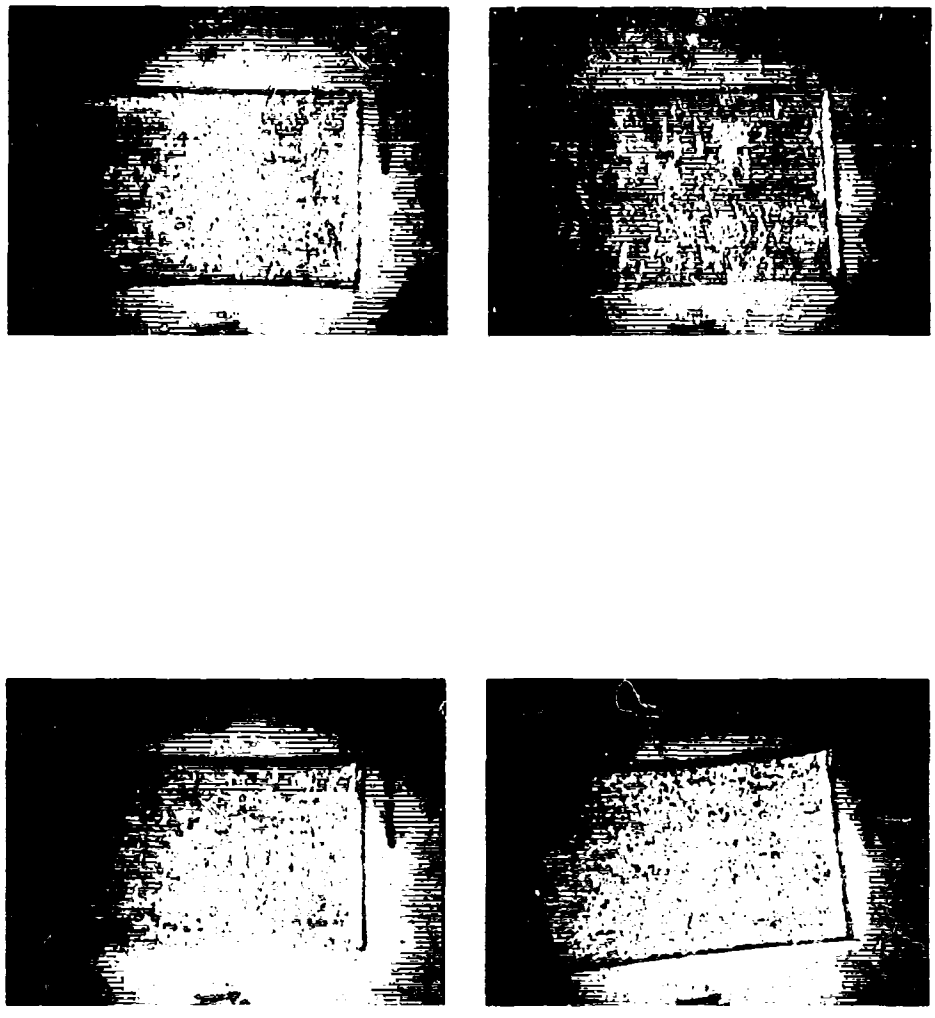
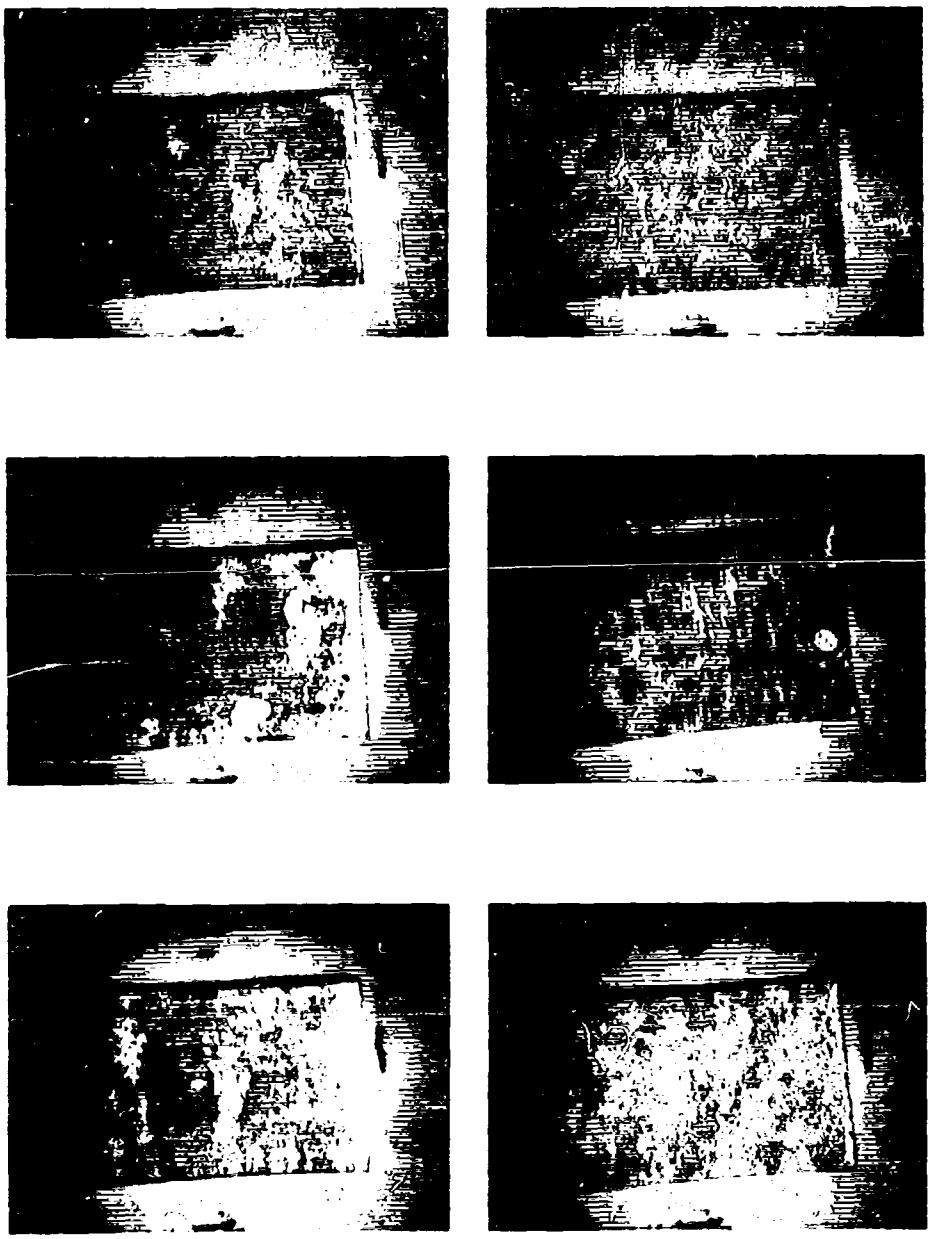
* The ferric ion at pH 7 will be converted to highly insoluble $Fe(OH)_3$.



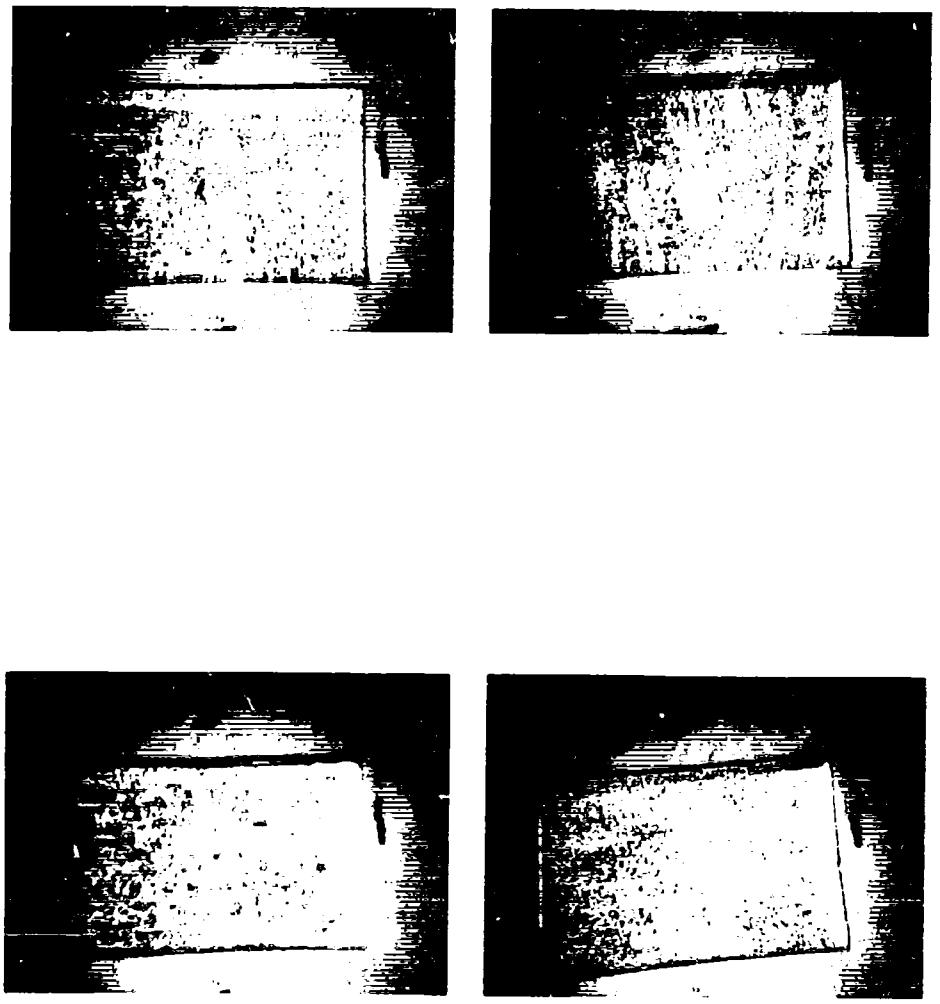
LEGEND: CONCENTRATIONS OF $CaCl_2$ FROM LEFT TO RIGHT: 8×10^{-6}M, 8×10^{-5}M, 8×10^{-4}M, 8×10^{-3}M, 8×10^{-2}M. ALLOY 7075 IS REPRESENTED IN THE TOP FIGURES AND 2024 IN THE BOTTOM ONES. THE ALLOYS WERE IMMERSED IN THE INDICATED SOLUTIONS 48 HOURS, pH 7.0, TEMPERATURE 30°C.

Figure 1. Corrosion of 2024 and 7075 Alloys by Varying Concentrations of $CaCl_2$ (Sheet 1 of 2)



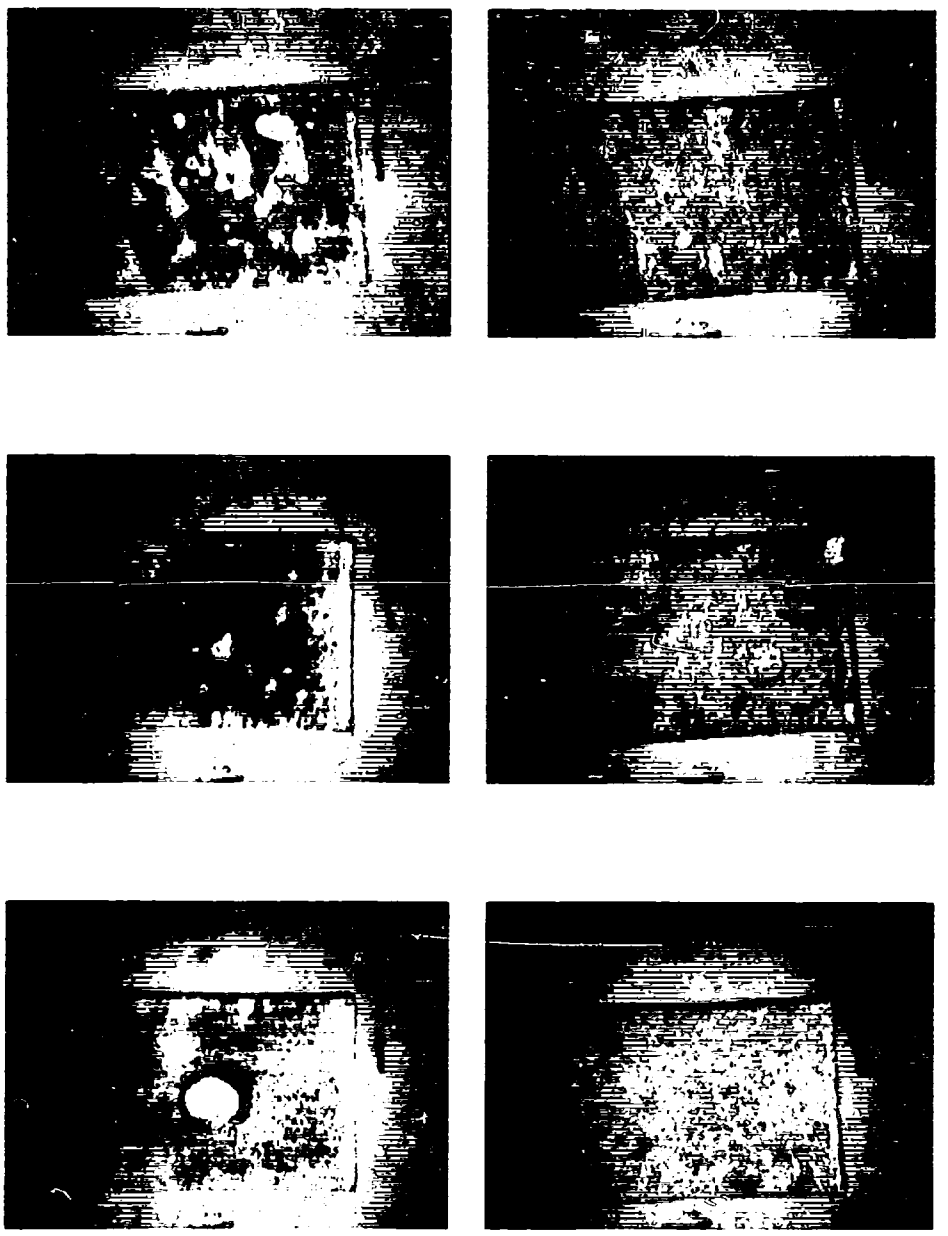
LEGEND: CONCENTRATIONS OF $CaCl_2$ FROM LEFT TO RIGHT: 8×10^{-6}M, 8×10^{-5}M, 8×10^{-4}M, 8×10^{-3}M, 8×10^{-2}M. ALLOY 7075 IS REPRESENTED IN THE TOP FIGURES AND 2024 IN THE BOTTOM ONES. THE ALLOYS WERE IMMERSED IN THE INDICATED SOLUTIONS 48 HOURS, pH 7.0, TEMPERATURE 30°C.

Figure 1. Corrosion of 2024 and 7075 Alloys by Varying Concentrations of $CaCl_2$ (Sheet 2 of 2)



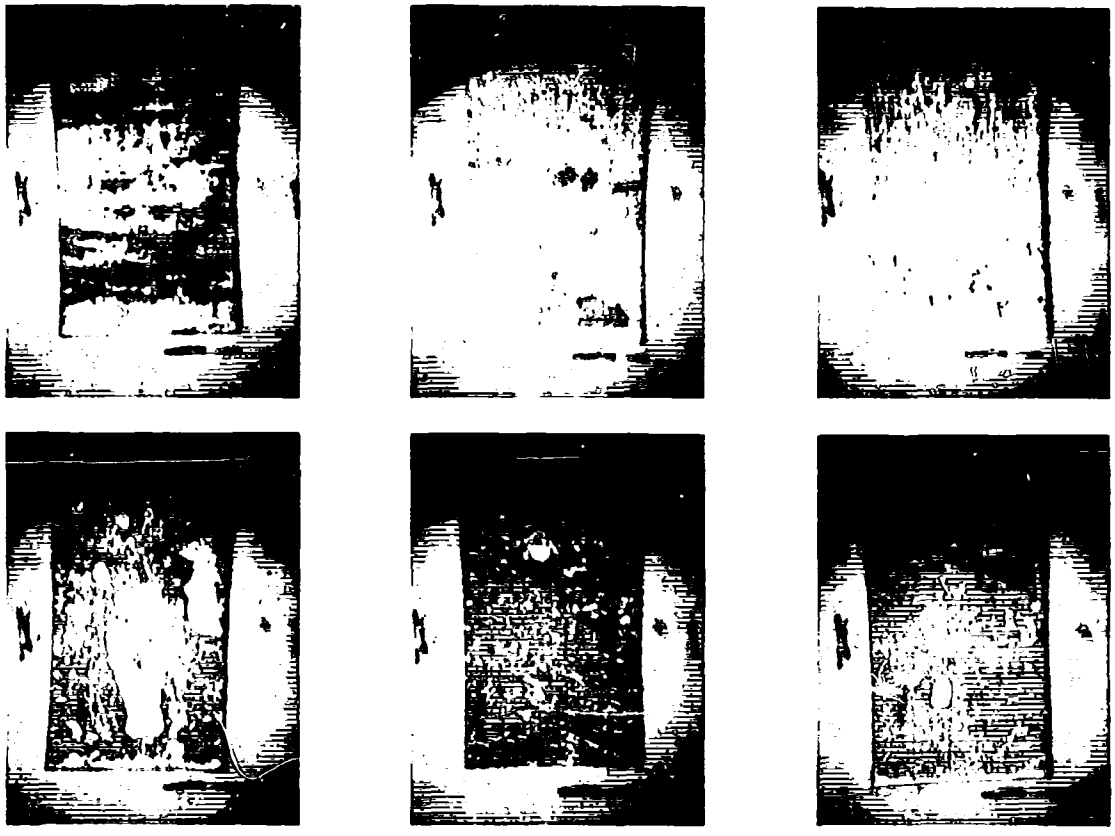
LEGEND: CONCENTRATIONS OF $FeCl_3$ FROM LEFT TO RIGHT: 8×10^{-6}M, 8×10^{-5}M, 8×10^{-4}M, 8×10^{-3}M, 8×10^{-2}M. ALUMINUM ALLOYS 7075 (TOP) AND 2024 (BOTTOM) WERE IMMERSED IN WATER-METAL SOLUTIONS, pH 7.0, TEMPERATURE 30°C FOR 48 HOURS.

Figure 2. Corrosion of 2024 and 7075 Alloys by Varying Concentrations of $FeCl_3$ (Sheet 1 of 2)



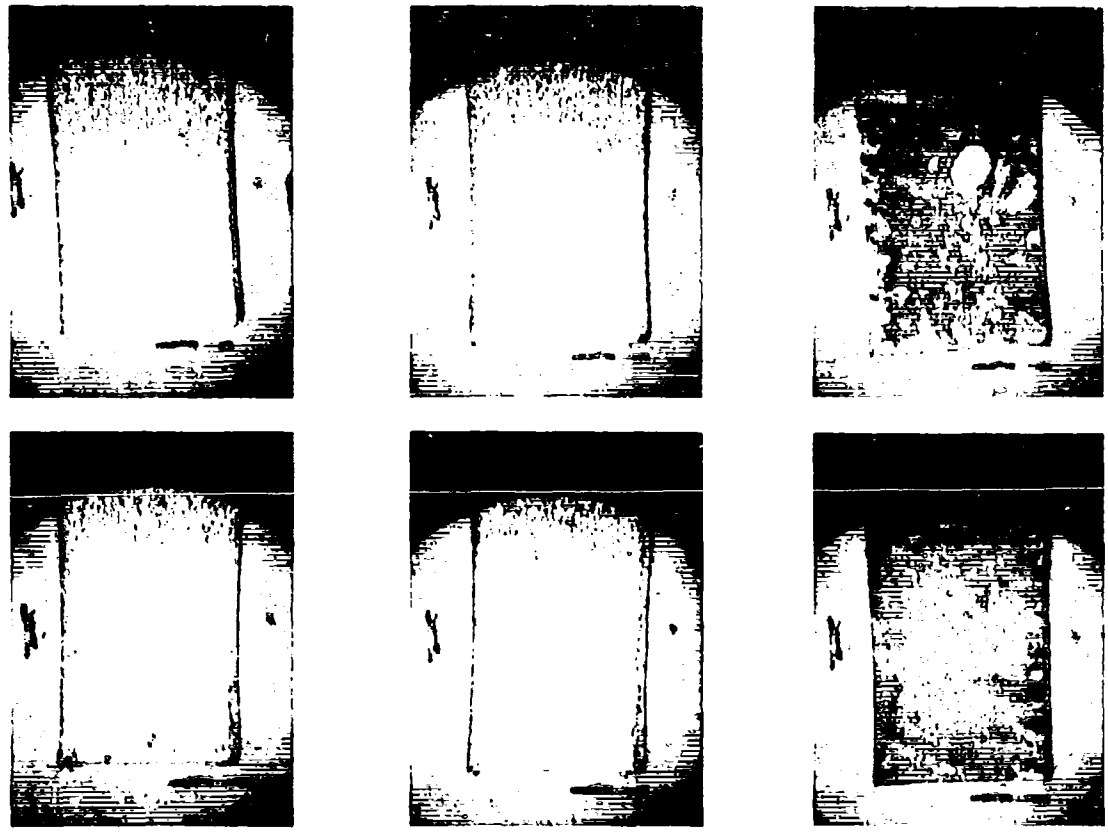
LEGEND: CONCENTRATIONS OF $FeCl_3$ FROM LEFT TO RIGHT: 8×10^{-6}M, 8×10^{-5}M, 8×10^{-4}M, 8×10^{-3}M, 8×10^{-2}M, ALUMINUM ALLOYS 7075 (TOP) AND 2024 (BOTTOM) WERE IMMERSED IN WATER-METAL SOLUTIONS, pH 7.0, TEMPERATURE 30°C FOR 48 HOURS.

Figure 2. Corrosion of 2024 and 7075 Alloys by Varying Concentrations of $FeCl_3$ (Sheet 2 of 2)



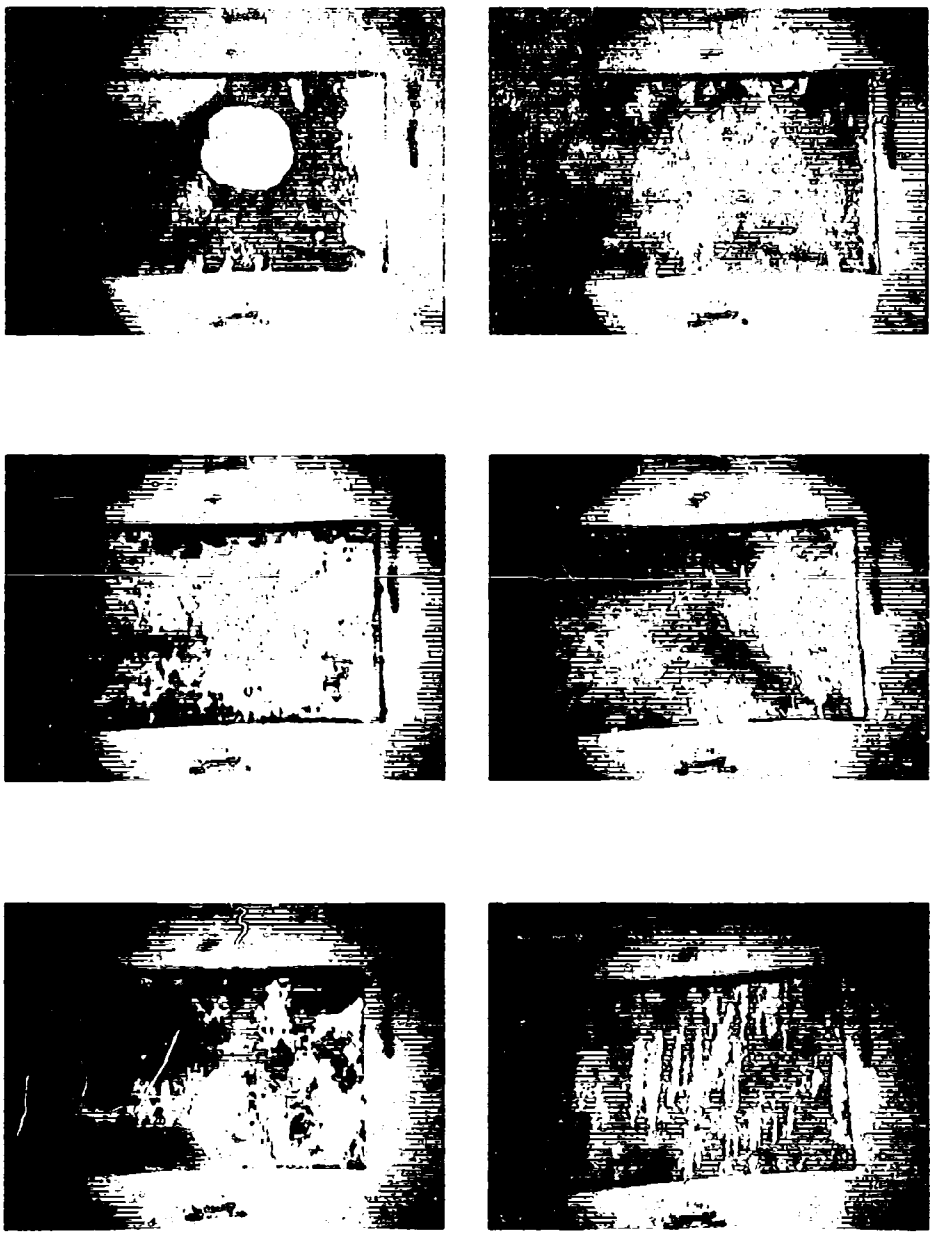
LEGEND: POTASSIUM NITRATE WAS ADDED TO 8×10^{-4} M $FeCl_3$ TO GIVE SOLUTIONS CONTAINING (FROM LEFT TO RIGHT) 1.2×10^{-6} M KNO_3, 1.2×10^{-5} M KNO_3, 1.2×10^{-4} M KNO_3, 1.2×10^{-3} M KNO_3, 1.2×10^{-2} M KNO_3, AND 0.0 M KNO_3. ALUMINUM ALLOYS 7075 (TOP ROW) AND 2024 (BOTTOM ROW) WERE IMMERSED IN THESE SOLUTIONS AT pH 7.0 AND 30°C FOR 48 HOURS.

Figure 3. Corrosion of 7075 and 2024 Alloys by 8×10^{-4} M $FeCl_3$ in Varying Concentrations of KNO_3 (Sheet 1 of 2)



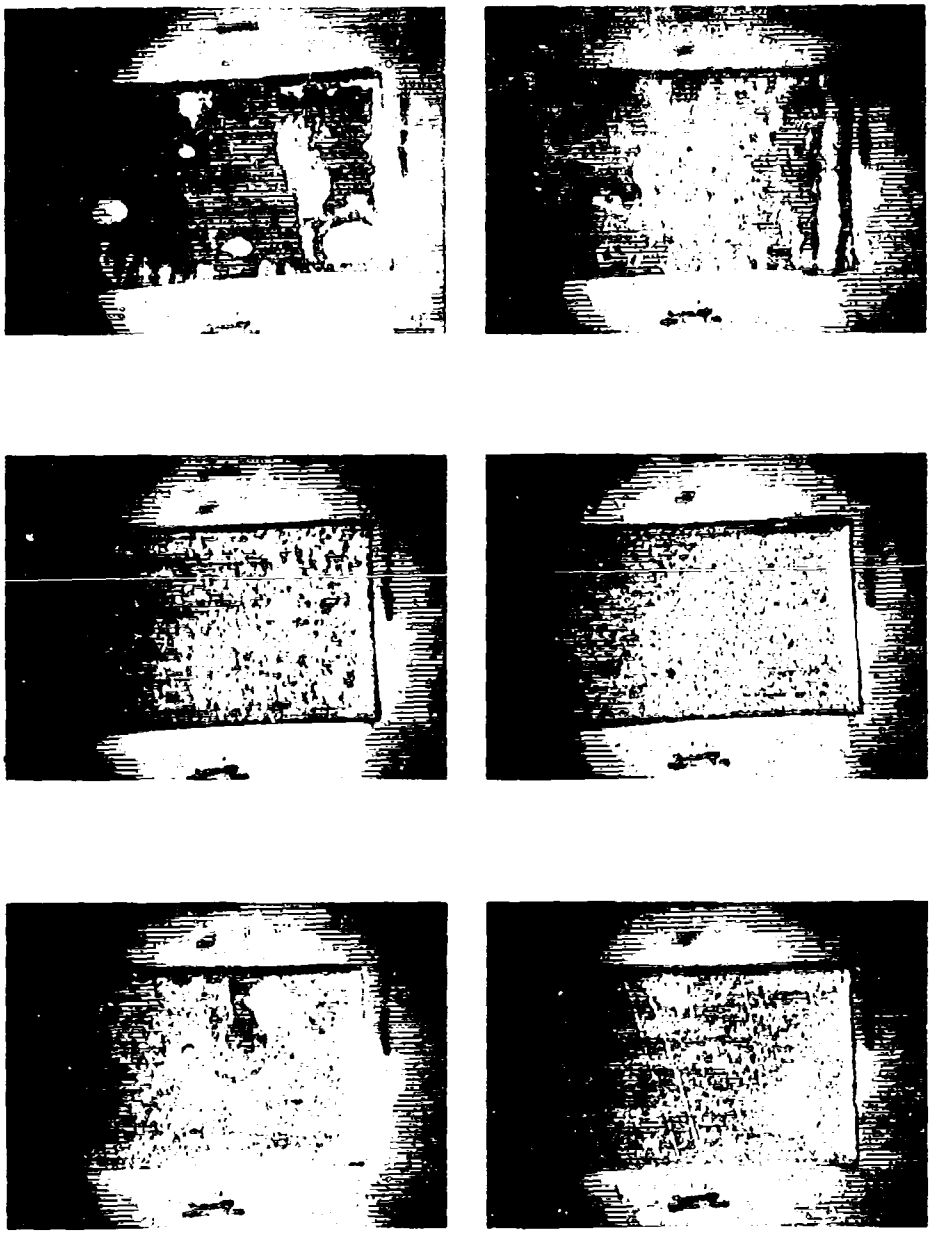
LEGEND: POTASSIUM NITRATE WAS ADDED TO 8 X 10^{-4} M $FeCl_3$ TO GIVE SOLUTIONS CONTAINING (FROM LEFT TO RIGHT) 1.2 X 10^{-6} M KNO_3, 1.2 X 10^{-5} M KNO_3, 1.2 X 10^{-4} M KNO_3, 1.2 X 10^{-3} M KNO_3, 1.2 X 10^{-2} M KNO_3, AND 0.0 M KNO_3. ALUMINUM ALLOYS 7075 (TOP ROW) AND 2024 (BOTTOM ROW) WERE IMMERSED IN THESE SOLUTIONS AT pH 7.0 AND 30°C FOR 48 HOURS.

Figure 3. Corrosion of 7075 and 2024 Alloys by 8 x 10^{-4} M $FeCl_3$ in Varying Concentrations of KNO_3 (Sheet 2 of 2)



LEGEND: 8 X 10^{-4} $NaCl_2$ - OTHER CONDITIONS AS INDICATED IN FIGURE 3

Figure 4. Corrosion of 7075 and 2024 Alloys by 8 x 10^{-4} M $CaCl_2$ in Varying Concentrations of KNO_3 (Sheet 1 of 2)



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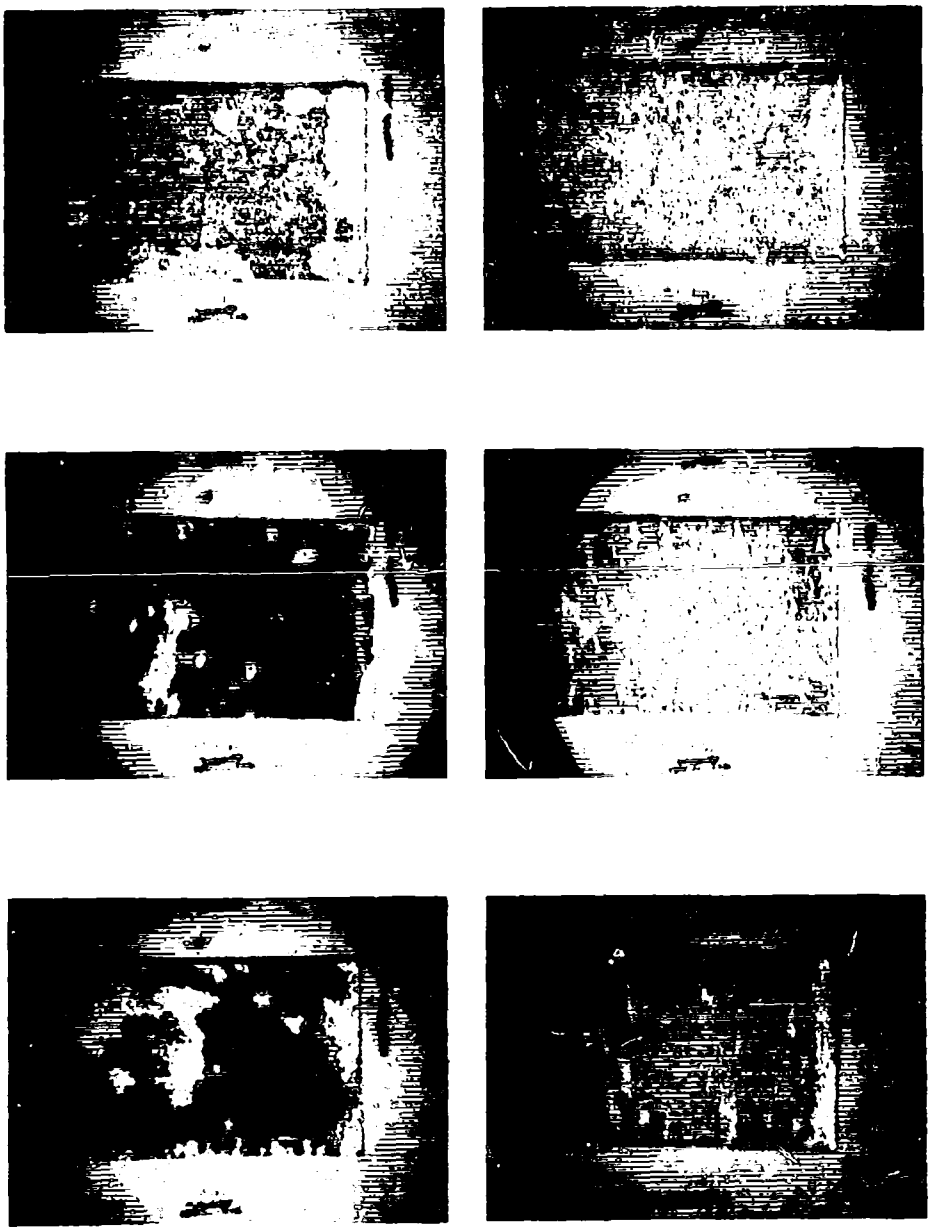
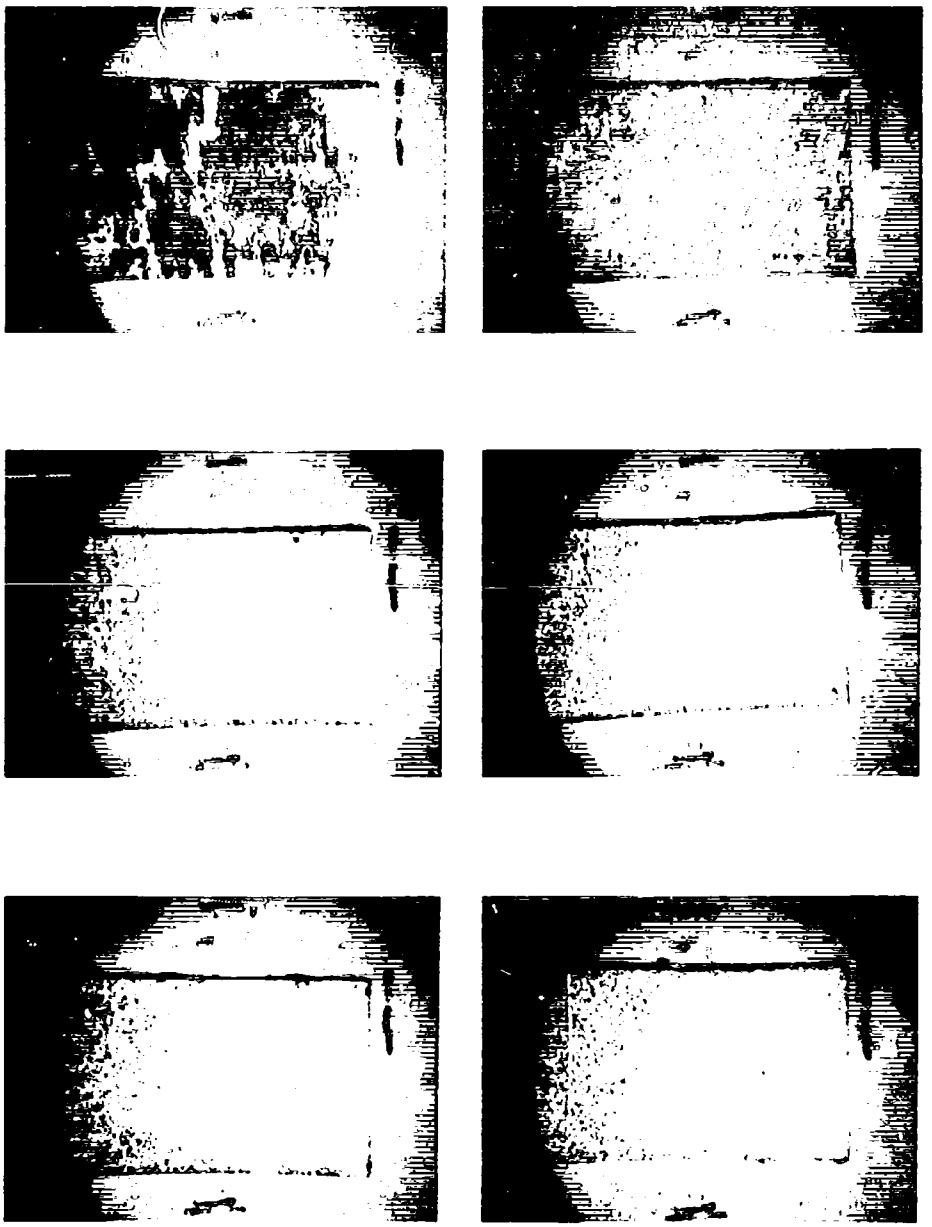


LEGEND: 8 X 10^{-4} M NaCl - OTHER CONDITIONS AS INDICATED IN FIGURE 3.

Figure 5. Corrosion of 7075 and 2024 Alloys by 8 x 10^{-4}M N_aCl in Varying Concentrations of KNO_3 (Sheet 1 of 2)



LEGEND: 8 X 10^{-4} M NaCl - OTHER CONDITIONS AS INDICATED IN FIGURE 3.

Figure 5. Corrosion of 7075 and 2024 Alloys by 8 x 10^{-4}M N_aCl in Varying Concentrations of KNO_3 (Sheet 2 of 2)

This concentration again was 0.06 g KNO_3 per liter or 5.9×10^{-4} KNO_3. These observations suggested that inhibition of corrosion depends on a stoichiometric relationship between the concentration of corrosion inhibitor and the compounds stimulating corrosion.

The chemical mechanisms by which nitrate acts as a passivator are unclear. It seems highly improbable that nitrate forms stable compounds with calcium or ferric ion and thereby prevents their participation in the corrosion process. It seems equally improbable that nitrate complexes with the aluminum surface and renders it resistant to the action or corrosion ions, but the ability of nitrate to inhibit corrosion appears to depend on its concentration relative to other elements of the growth medium. Therefore, microbial activities which remove nitrate from a medium more rapidly than iron or calcium tend to make the medium more corrosive for aluminum.

Orthophosphate is known to be an inhibitor of corrosion, and like some of the ions which stimulate corrosion, it is essential for microbial growth and respiration.

The concentrations of phosphate inhibiting the aluminum corrosion caused by 8×10^{-4} Fe Cl_3*, $CaCl_2$ and NaCl were shown respectively by the pictures in Figures 6, 7 and 8. Phosphate, like nitrate, inhibited corrosion approximately 50% in 5.9×10^{-4} M solution.

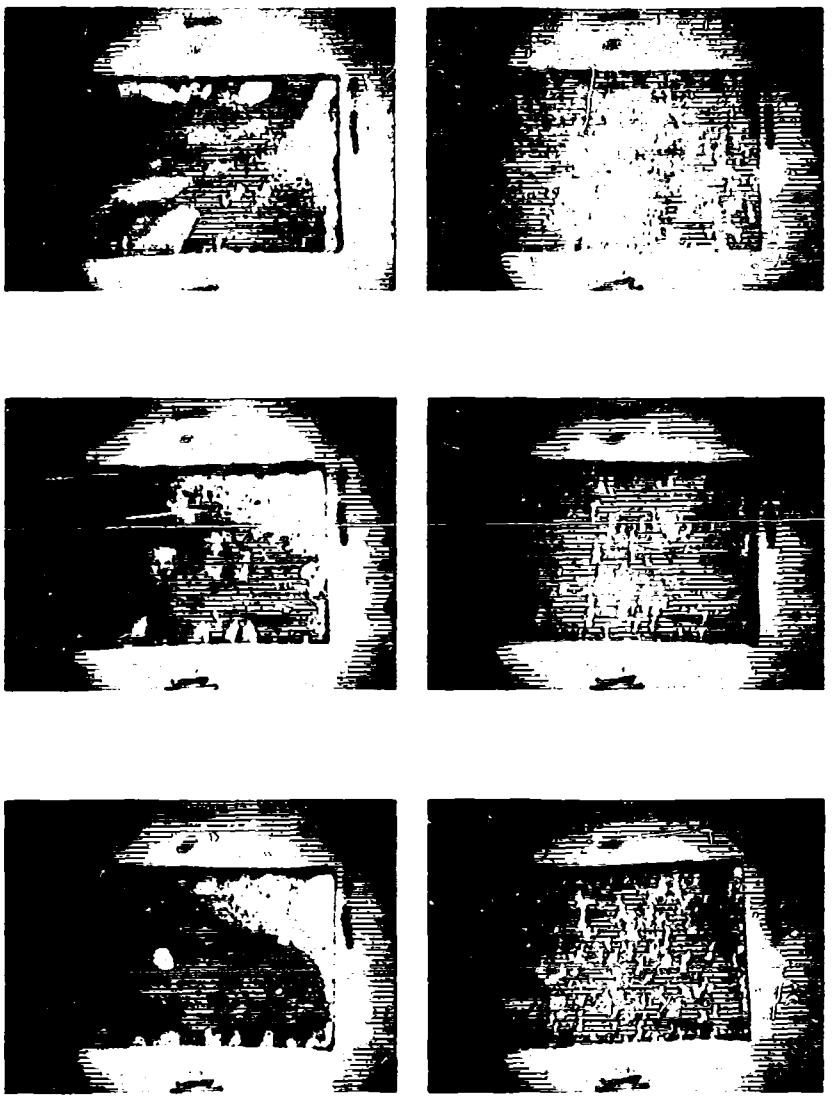
2. Aluminum Corrosion Caused by Removal of Nitrate from Bushnell-Haas Medium During Growth of Fuel Microorganisms.

A knowledge of certain features of microbial physiology suggested a biological mechanism by which the growth of organisms could cause aluminum corrosion. Microorganisms that grow on mineral media, such as those which oxidize fuel, utilize the various components of mineral medium at different rates and to different extents. The quantity of iron or calcium used for growth is far less than the quantity of phosphate taken up by the cell, which is itself less than the quantity of nitrate used in the formation of cell protein and nucleic acids. It appeared, therefore, that the growth of microorganisms in media low in nitrate and phosphate would result in the removal of these radicals while the calcium, iron, and chloride present would remain in sufficient concentration to cause aluminum corrosion.

Tests were performed to determine whether organisms would remove nitrate from the growth medium and allow the corrosive ions to act on aluminum.

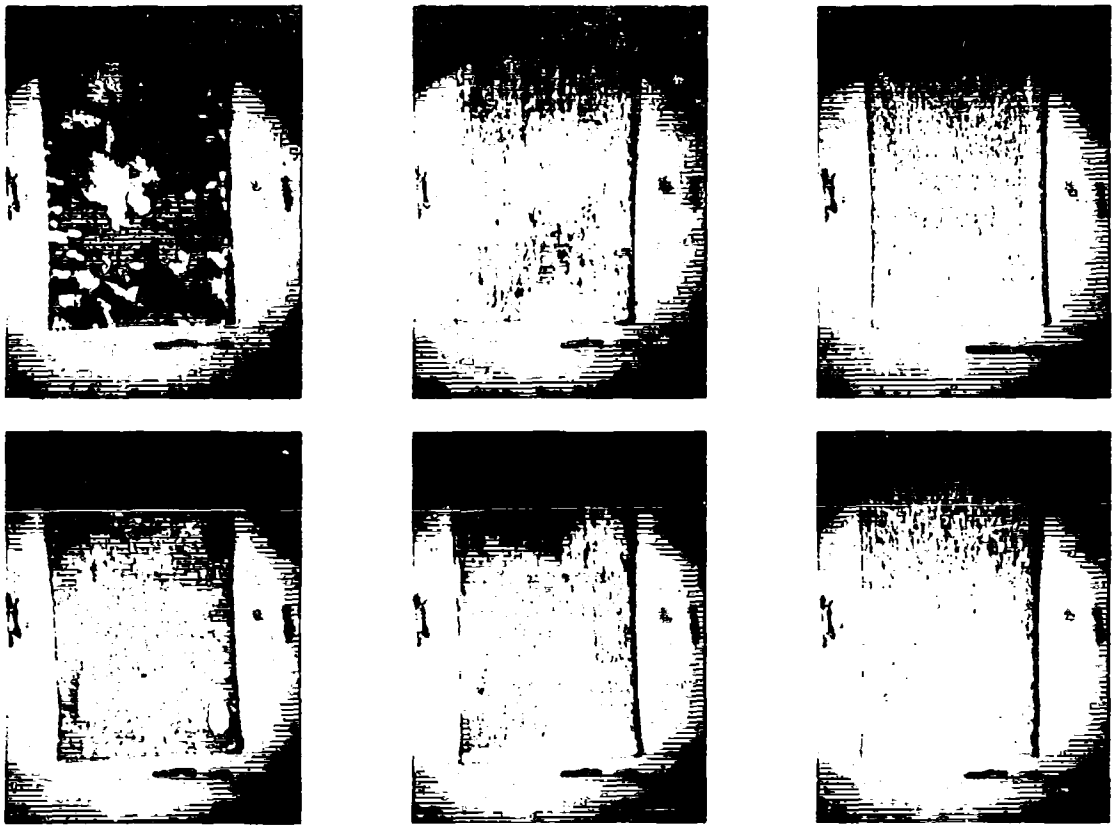
In this study, two media have been found to be especially useful in the growth of organisms on a fuel overlay. These were the Bushnell-Haas medium and a medium recently formulated by Sharpley Laboratories, Inc. Both media have ammonium nitrate as thesource of nitrogen, but as the data show, nitrate ion was an especially strong inhibitor of aluminum corrosion. Tests were set

* The ferric ion at pH 7 will be converted to highly insoluble $Fe(OH)_3$



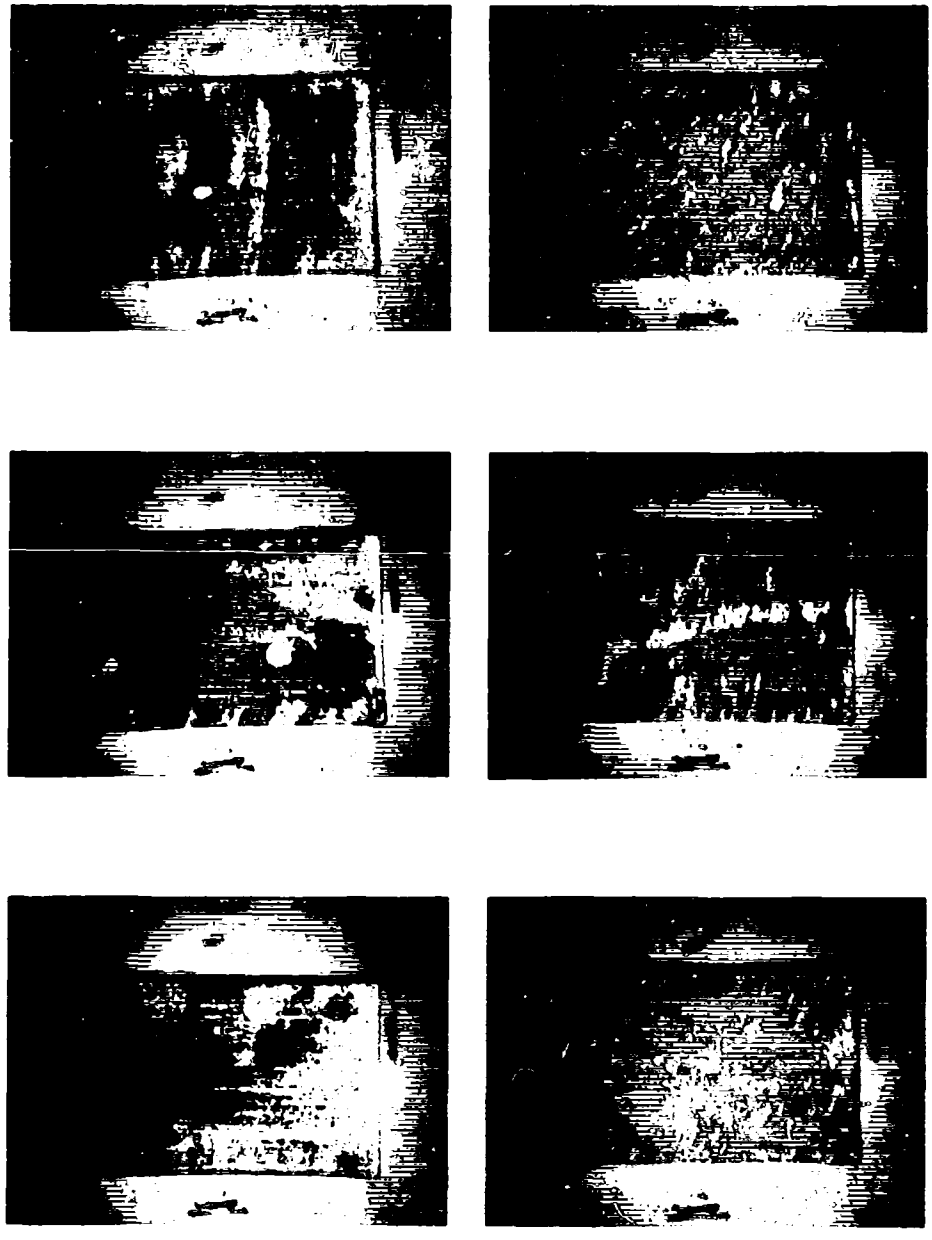
LEGEND: DIBASIC POTASSIUM PHOSPHATE WAS ADDED TO 8 X 10^{-4}M SOLUTIONS OF $FeCl_3$. THE FIGURES FROM LEFT TO RIGHT REPRESENT 0 (8 X 10^{-4} M $FeCl_3$ ONLY), 1.1 X 10^{-6}M, 1.1 X 10^{-5}M, 1.1 X 10^{-4}M, 1.1 X 10^{-3} AND 1.1 X 10^{-2}M SOLUTION OF K_2HPO_4 PLUS 8 X 10^{-4} M $FeCl_3$. ALLOYS 7075 (TOP ROW) AND 2024 (BOTTOM ROW) WERE IMMERSED IN THESE SOLUTIONS, pH 7.0 AT 30°C FOR 48 HOURS.

Figure 6. Corrosion of 7075 and 2024 Alloys by 8 x 10^{-4} M $FeCl_3$ in Solutions Containing Varying Concentrations of K_2HPO_4 (Sheet 1 of 2)



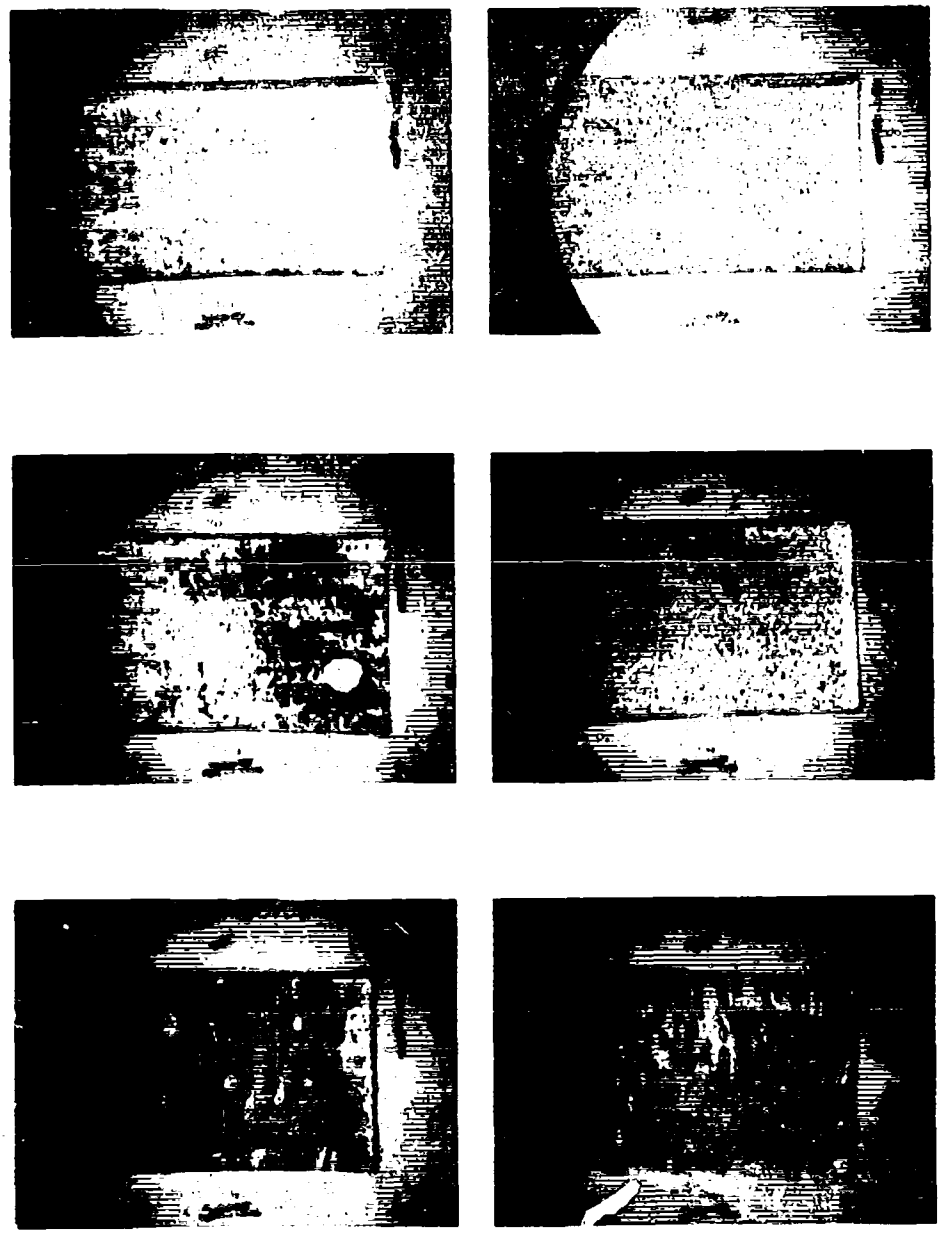
LEGEND: DIBASIC POTASSIUM PHOSPHATE WAS ADDED TO 8 X 10^{-4} M SOLUTIONS OF $FeCl_3$. THE FIGURES FROM LEFT TO RIGHT REPRESENT 0 (8 X 10^{-4} M $FeCl_3$ ONLY), 1.1 X 10^{-6}M, 1.1 X 10^{-5}M, 1.1 X 10^{-4}M, 1.1 X 10^{-3}M AND 1.1 X 10^{-2}M SOLUTION OF K_2HPO_4 PLUS 8 X 10^{-4}M $FeCl_3$. ALLOYS 7075 (TOP ROW) AND 2024 (BOTTOM ROW) WERE IMMERSED IN THESE SOLUTIONS, pH 7.0 AT 30°C FOR 48 HOURS.

Figure 6. Corrosion of 7075 and 2024 Alloys by 8 x 10^{-4} M $FeCl_3$ in Solutions Containing Varying Concentrations of K_2HPO_4 (Sheet 2 of 2)



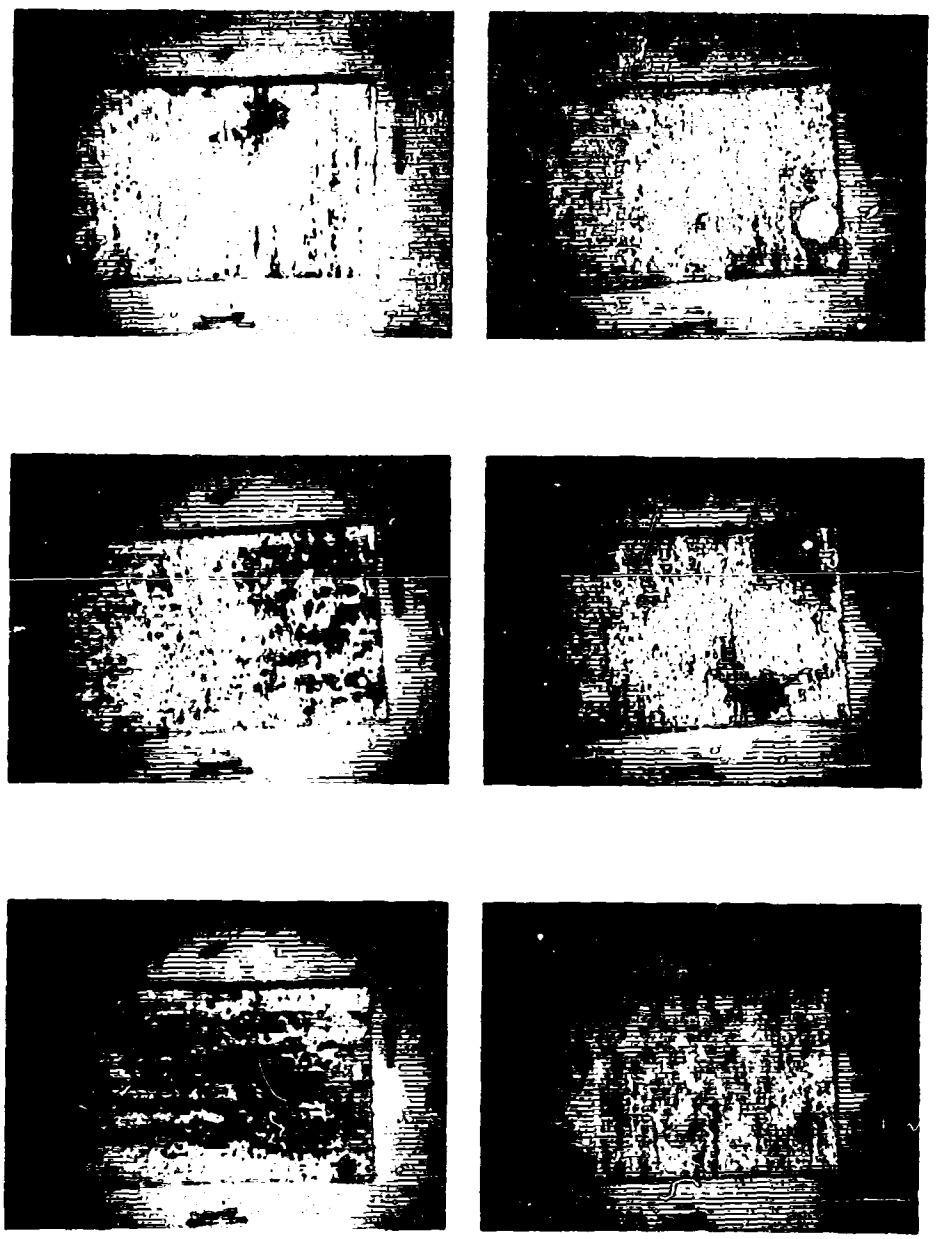
LEGEND: 8 X 10^{-4} M $CaCl_2$ - OTHER CONDITIONS AS INDICATED IN FIGURE 6

Figure 7. Corrosion of 7075 and 2024 Alloys by 8 x 10^{-4} M $CaCl_2$ in Solutions Containing Varying Concentrations of K_2HPO_4 (Sheet 1 of 2)



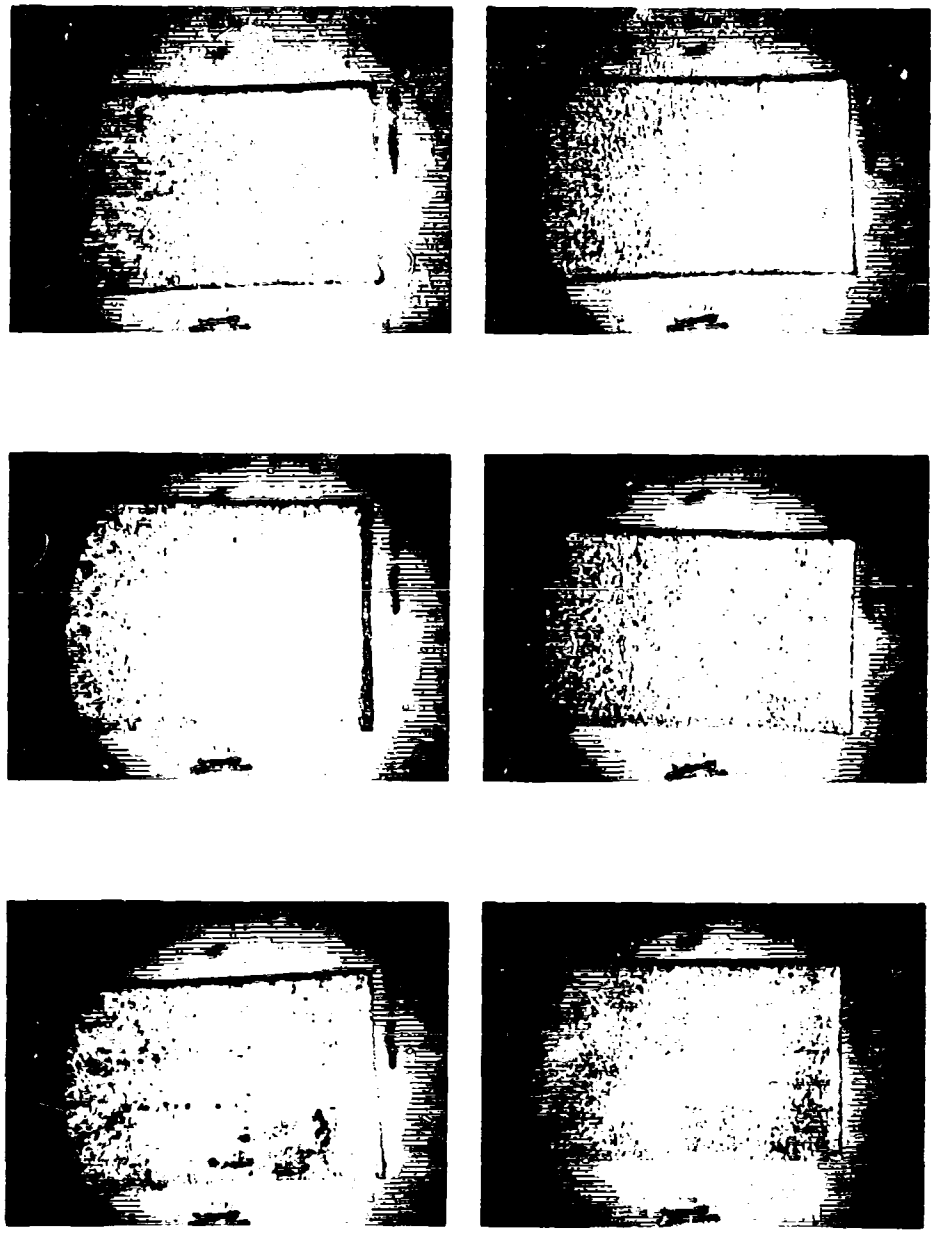
LEGEND: 8 X 10^{-4} M $CoCl_2$ - OTHER CONDITIONS AS INDICATED IN FIGURE 6

Figure 7. Corrosion of 7075 and 2024 Alloys by 8 x 10^{-4} M $CaCl_2$ in Solutions Containing Varying Concentrations of K_2HPO_4 (Sheet 2 of 2)



LEGEND: 8 X 10^{-4} M NaCl. OTHER CONDITIONS AS INDICATED IN FIGURE 6

Figure 8. Corrosion of 7075 and 2024 Alloys by 8 x 10^{-4} M NaCl in Solutions Containing Varying Concentrations of K_2HPO_4 (Sheet 1 of 2)



LEGEND: 8 X 10^{-4}M NaCl - OTHER CONDITIONS AS INDICATED IN FIGURE 6

Figure 8. Corrosion of 7075 and 2024 Alloys by 8 x 10^{-4} M NaCl in Solutions Containing Varying Concentrations of K_2HPO_4
(Sheet 2 of 2)

up to determine (1) the ability of a mixed culture of fuel organisms to grow on media containing only NO_3^- as a nitrogen source, and (2) the yield of cells obtained at various NO_3^- concentrations. If nitrate alone were utilizable as a nitrogen source, then tests could be subsequently performed to find out if fuel organisms fixed nitrate in the cell body in such a manner and in such quantity that it could no longer act as a corrosion inhibitor.

Organisms grown on fuel were washed in distilled water and used as inocula in media, all of which contained the salts of the Bushnell-Haas medium, but without ammonium ion and at various concentrations of KNO_3. The concentrations of nitrate chosen were those known to be below and above levels inhibiting aluminum corrosion by calcium sulfate and iron sulfate at the concentrations of the BH medium. During various growth periods, the yield of variable organisms depended on the nitrate concentration in the range of concentrations employed (Table 1). This dependence showed that nitrate, rather than the other ions of the medium, was the growth-limiting factor, and this in turn suggested that nitrate had been used up by the organisms in those cultures where growth was below the maximum growth obtained. In the absence of added nitrate and ammonium ion neither growth occurred nor were the cells killed at a measurable rate.

Thus, it was established that organisms utilizing fuel as a carbon source could utilize nitrate as a nitrogen source, and that the concentration of nitrate which suppressed corrosion would support growth. The hypothesis that growth of bacteria at these nitrate concentrations would remove nitrate from solution in sufficient quantities that the ions which are essential to growth would cause the corrosion of aluminum was tested. Alternately, the removal of this radical from the growth medium would permit the uninhibited attack of bacterial products that were corrosive.

Modified Bushnell-Haas media were prepared which contained nitrate at various concentrations as the only source of nitrogen. Thirty identical flasks were prepared at each of several nitrate concentrations and coupons of both 2024 and 7075 alloy were placed in each flask. Half of the identical series was inoculated with a mixed culture of fuel-oxidizing organisms and the other half was maintained as a control of microbial contamination and aluminum corrosion.

The nitrate concentrations in these flasks were 0, 0.02, 0.04, 0.06, 0.08 and 1.2 g of KNO_3 per liter. A Bushnell-Haas medium with $(NH_4)_2 SO_4$ substituted for $(NH_4) NO_3$ was included. The flasks were incubated at 28° and periodically analyzed for corrosion and cell growth. In the absence of an added nitrogen source, neither growth nor appreciable killing was observed.

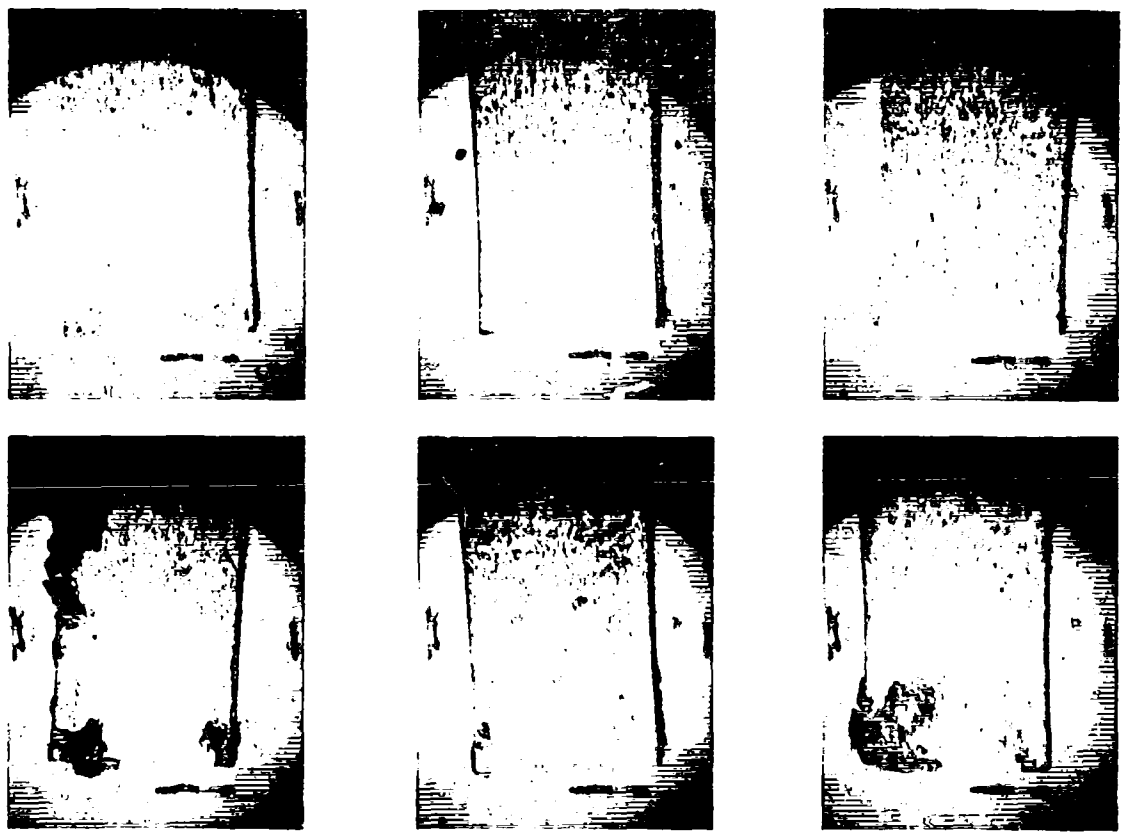
The extent of corrosion caused by microbial growth over various periods of time at different nitrate concentrations is shown by the pictures in Figures 9 through 18. The coupons photographed were chosen at random from fifteen replicas of inoculated and uninoculated media. The cupons are characteristic of the response of the entire group, thus reproducibility in all cases was high. The coupons were 1/4-inch wide and the photographic presentation gives a sevenfold magnification.

TABLE 1.

GROWTH OF A MIXED CULTURE IN MEDIA CONTAINING VARYING CONCENTRATION OF KNO_3

Day	Concentration of KNO_3 g/l						
	0g	0.02g	0.04g	0.06g	0.08g	1.2g	NBH
0	8×10^7	8.1×10^7	6.4×10^7	8.6×10^7	2.2×10^7	7×10^7	1.4×10^8
1	6.2×10^7	1.6×10^8	4.3×10^8	3.2×10^8	7.1×10^7	3×10^8	9.3×10^8
2	5.4×10^7	1.4×10^8	2.7×10^8	2.8×10^8	2.3×10^8	1×10^9	4×10^8
5	2×10^8	3.7×10^8	2.2×10^8	3.7×10^8	3×10^8	1.1×10^9	3.2×10^8
6	4.3×10^7	1.2×10^8	2.4×10^8	3.3×10^8	3.1×10^8	7.1×10^8	3.4×10^8
8	7×10^7	1.6×10^8	2.7×10^8	3.5×10^8	3.4×10^8	4.5×10^8	1.7×10^8
12	3×10^7	1.2×10^8	2.3×10^8	3.9×10^8	2.3×10^8	1.3×10^8	1×10^8
23	6×10^7	1.6×10^8	2.5×10^8	2.8×10^8	2.2×10^8	4×10^8	1×10^8
33	5.5×10^7	1.1×10^8	1.5×10^8	2.8×10^8	2.2×10^8	$1.x 10^8$	1.7×10^8

The composition of the above media was the same as that of NBH except that ammonium sulfate was replaced by KNO_3. Inoculum consisted of a mixed culture which had been grown in fuel-NBH and harvested by washing 3 times in distilled water. Each test flask received 5 ml of inocula.



LEGEND: FLASKS WERE INOCULATED WITH 5 ML OF MIXED CULTURE WHICH HAD BEEN GROWN 48 HOURS IN NBH-FUEL, HARVESTED BY CENTRIFUGATION AND WASHED 3 TIMES IN DISTILLED H_2O. AFTER INOCULATION THE FLASKS WERE PLACED AT 37°C FOR 20 DAYS ON A NEW BRUNSWICK SHAKER. THE TOP FIGURES REPRESENT UNINOCULATED CONTROL FLASKS CONTAINING SOLUTIONS, FROM LEFT TO RIGHT, OF 0, 0.02, 0.04, 0.06, 0.08, AND 1.2 GMS KNO_3 PER LITER AND THE LAST SOLUTION CONTAINING 1.0 GM OF $(NH_4)_2SO_4$ PER LITER. INOCULATED TEST FLASKS ARE REPRESENTED BY THE BOTTOM ROW OF FIGURES.

Figure 9. Corrosion of 7075 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 1 of 2)

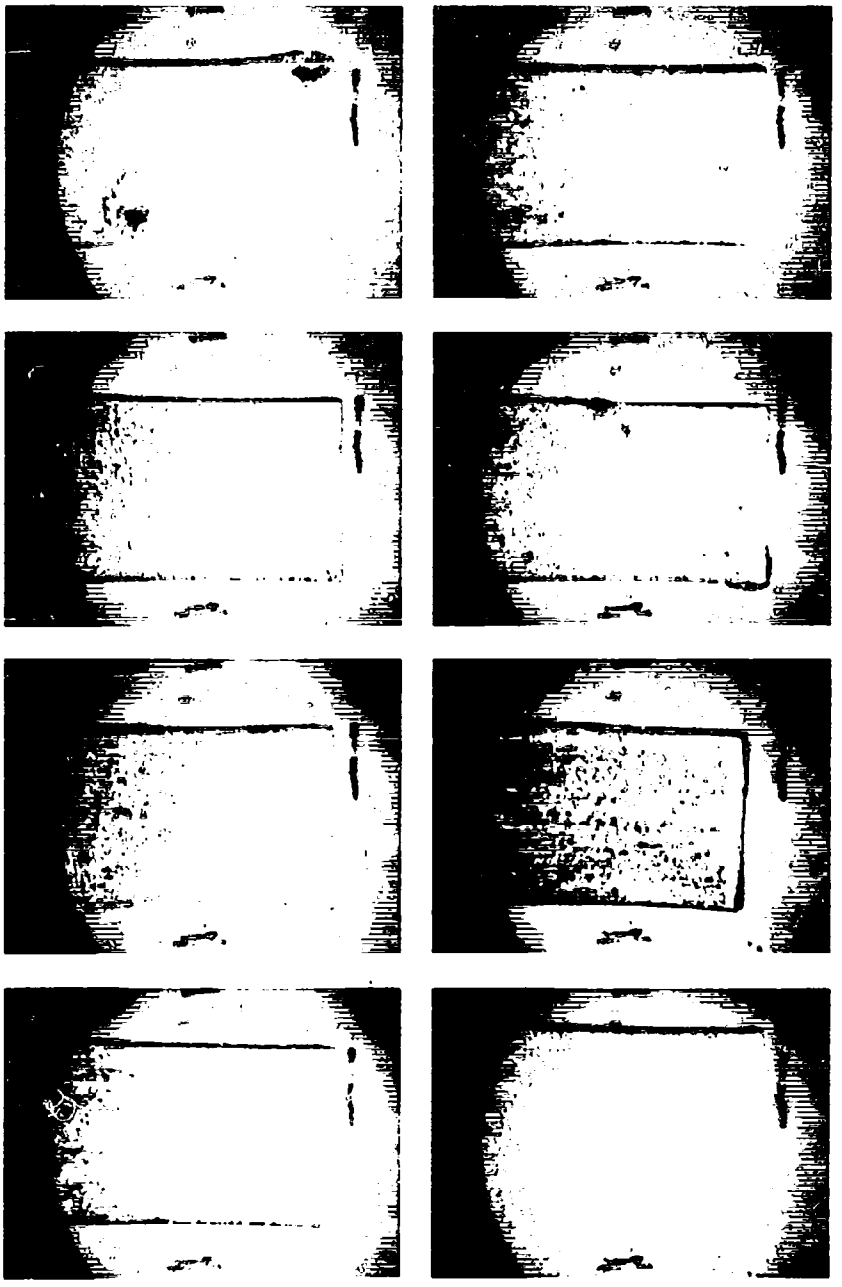


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Figure 9. Corrosion of 7075 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 2 of 2)

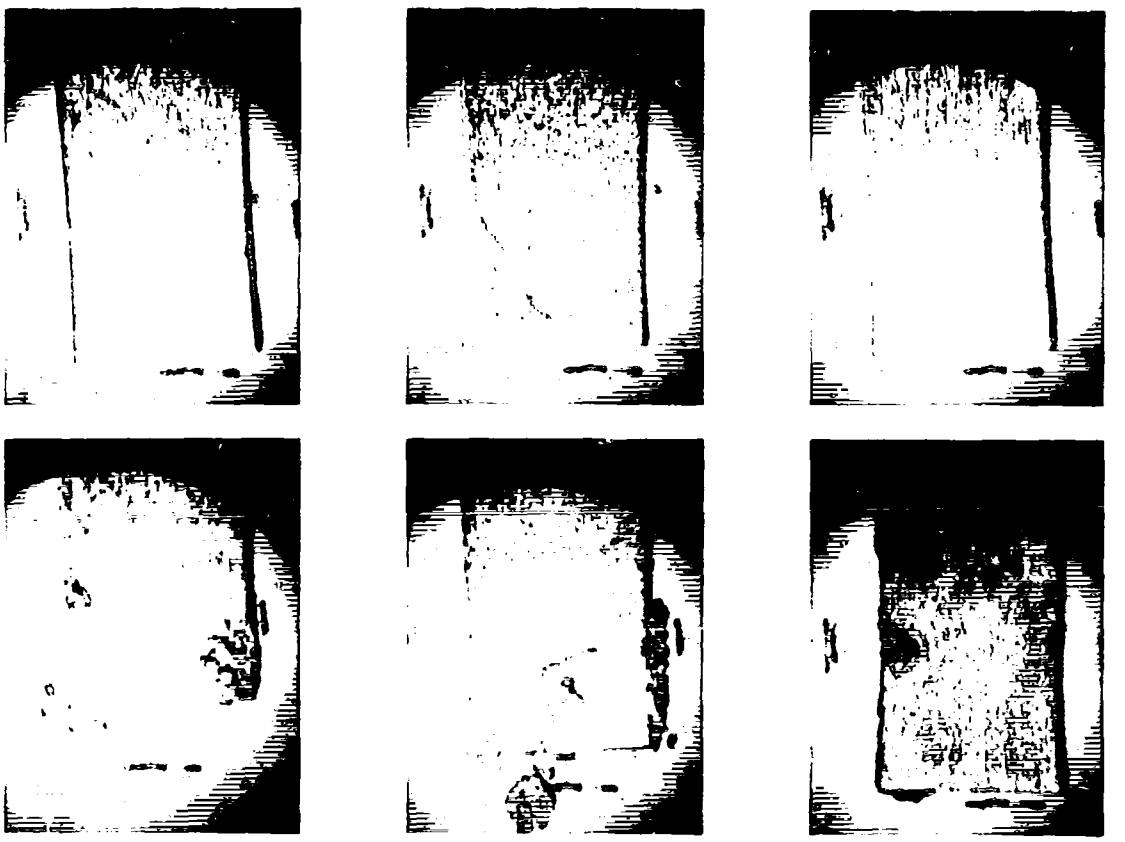

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Figure 10. Corrosion of 7075 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 1 of 2)

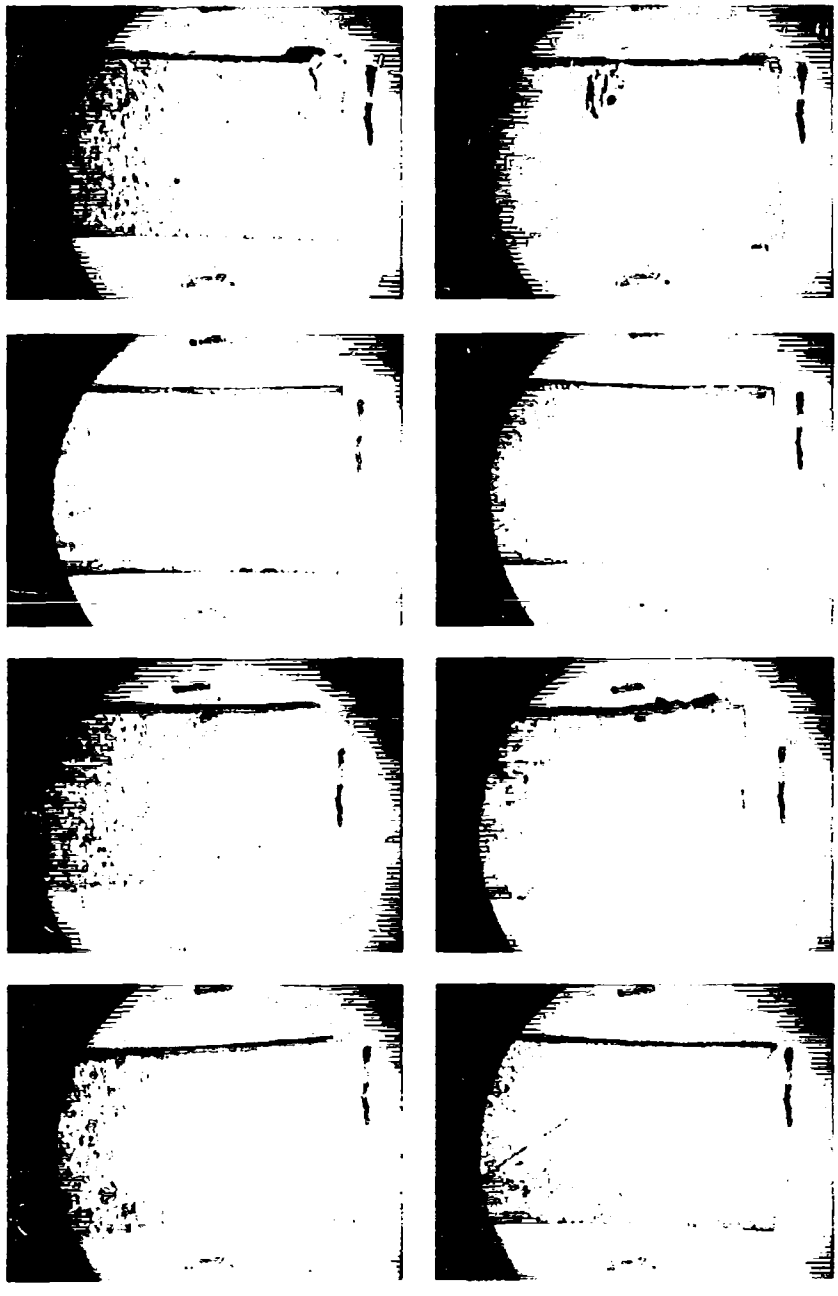

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Figure 10. Corrosion of 7075 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 2 of 2)

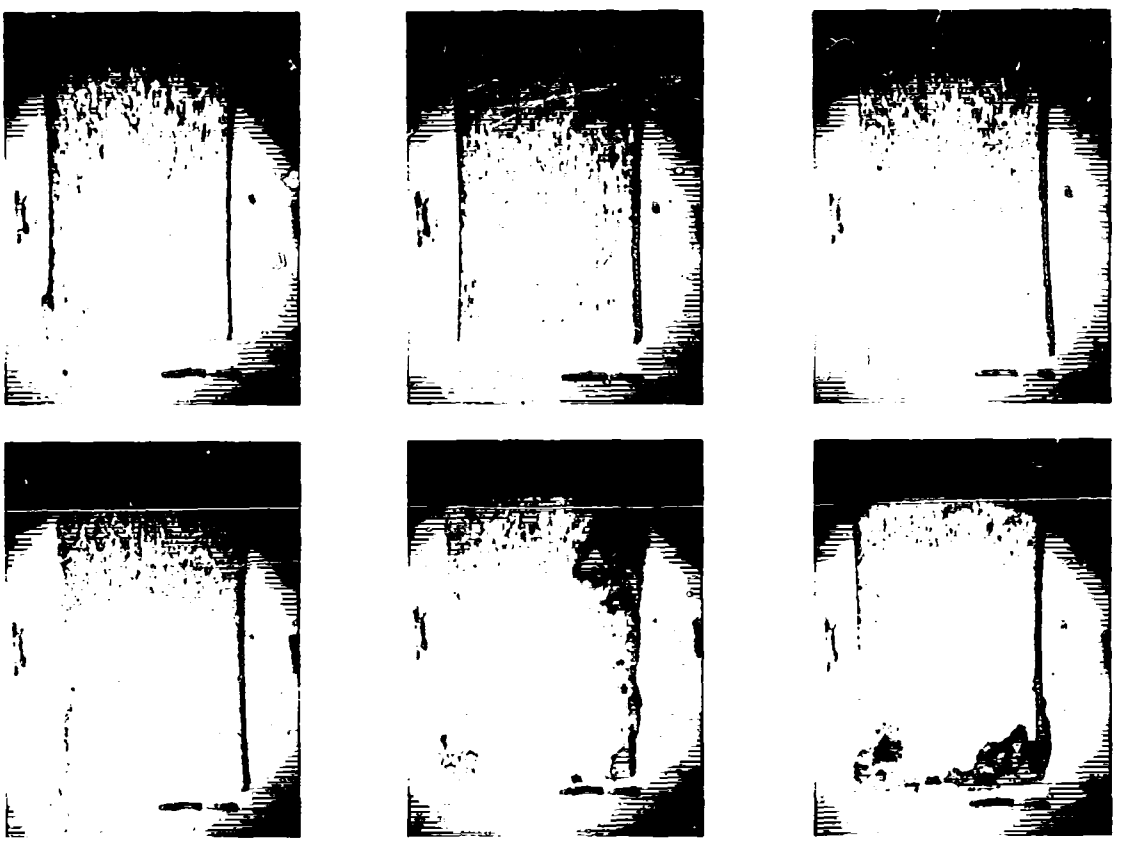

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Figure 11. Corrosion of 7075 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 1 of 2)

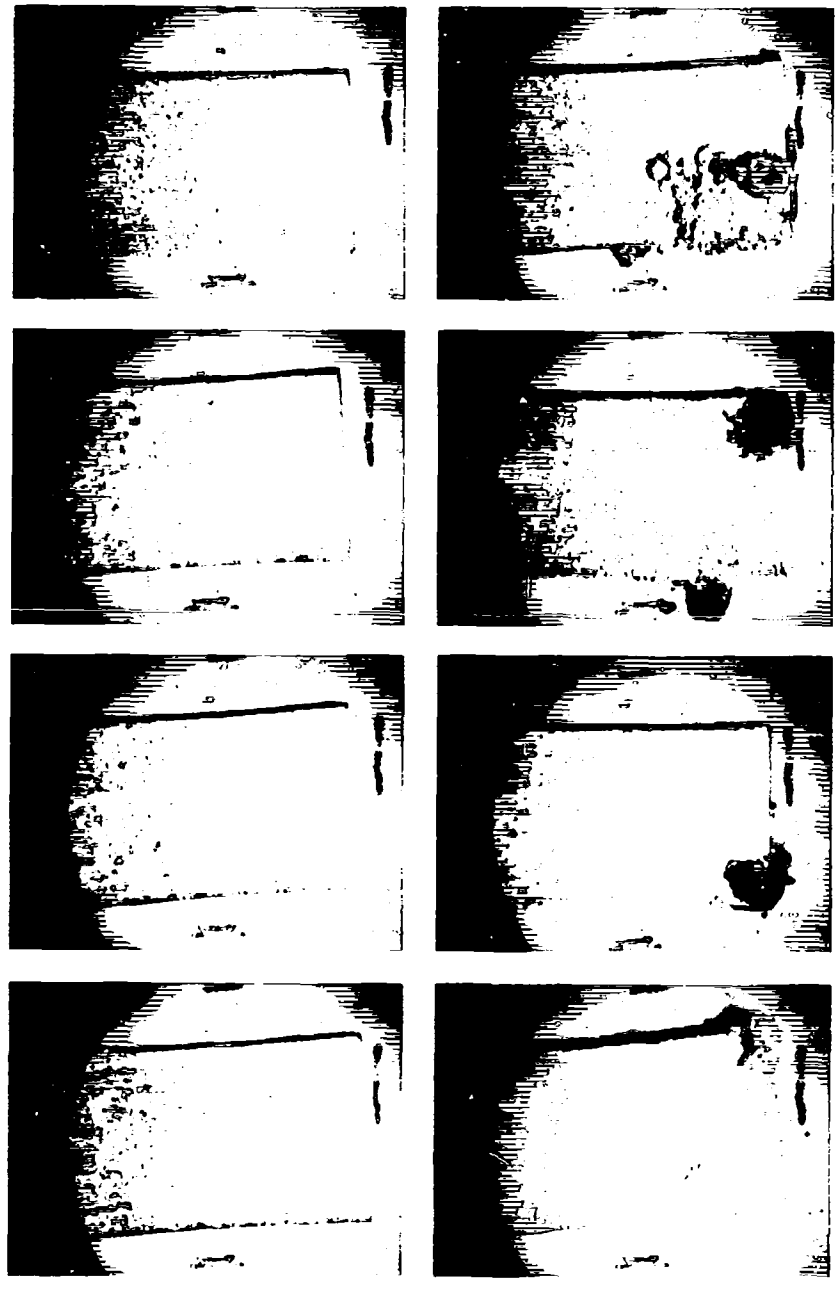

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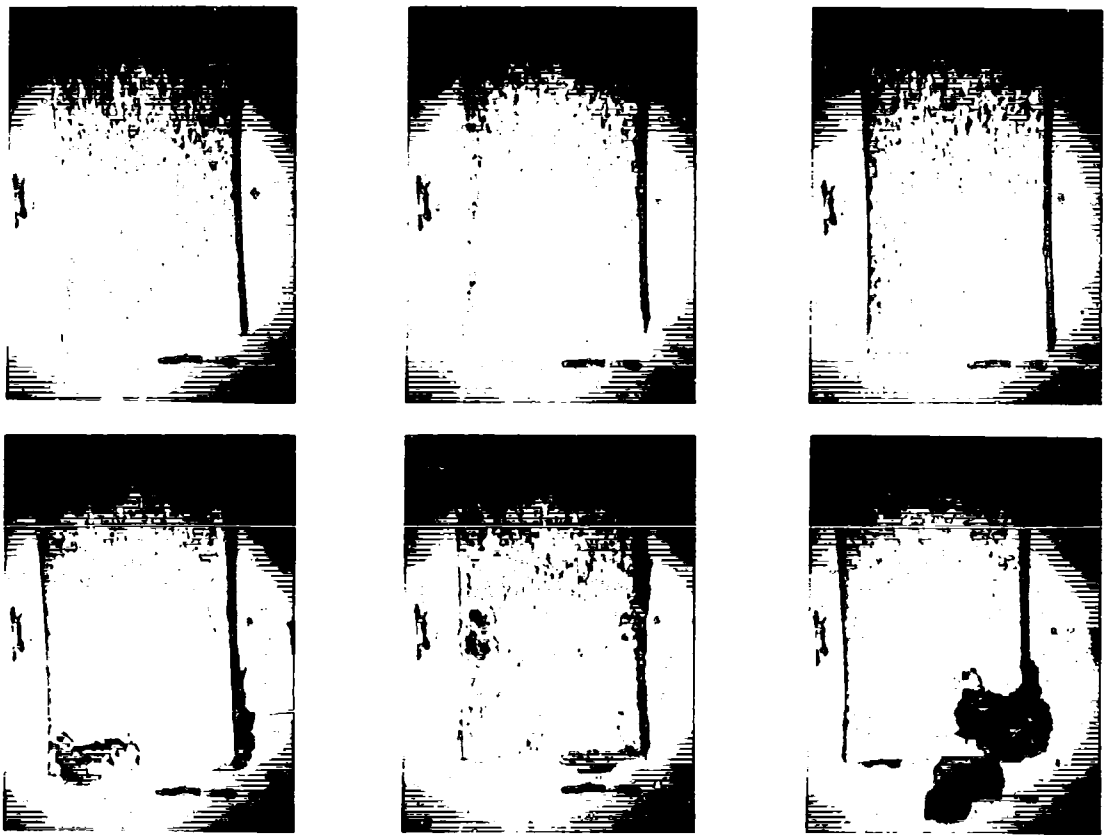
Figure 11. Corrosion of 7075 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 2 of 2)



LEGEND: FLASKS WERE INOCULATED WITH 5 ML OF MIXED CULTURE WHICH HAD BEEN GROWN 48 HOURS IN NBH-FUEL, HARVESTED BY CENTRIFUGATION AND WASHED 3 TIMES IN DISTILLED H_2O. AFTER INOCULATION THE FLASKS WERE PLACED AT 37°C FOR 80 DAYS ON A NEW BRUNSWICK SHAKER. THE TOP FIGURES REPRESENT UNINOCULATED CONTROL FLASKS CONTAINING SOLUTIONS, FROM LEFT TO RIGHT, OF 0, 0.02, 0.04, 0.06, 0.08, AND 1.2 GMS KNO_3 PER LITER AND THE LAST SOLUTION CONTAINING 1.0 GM OF $(NH_4)_2SO_4$ PER LITER. INOCULATED TEST FLASKS ARE REPRESENTED BY THE BOTTOM ROW OF FIGURES.

Figure 12. Corrosion of 7075 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 1 of 2)

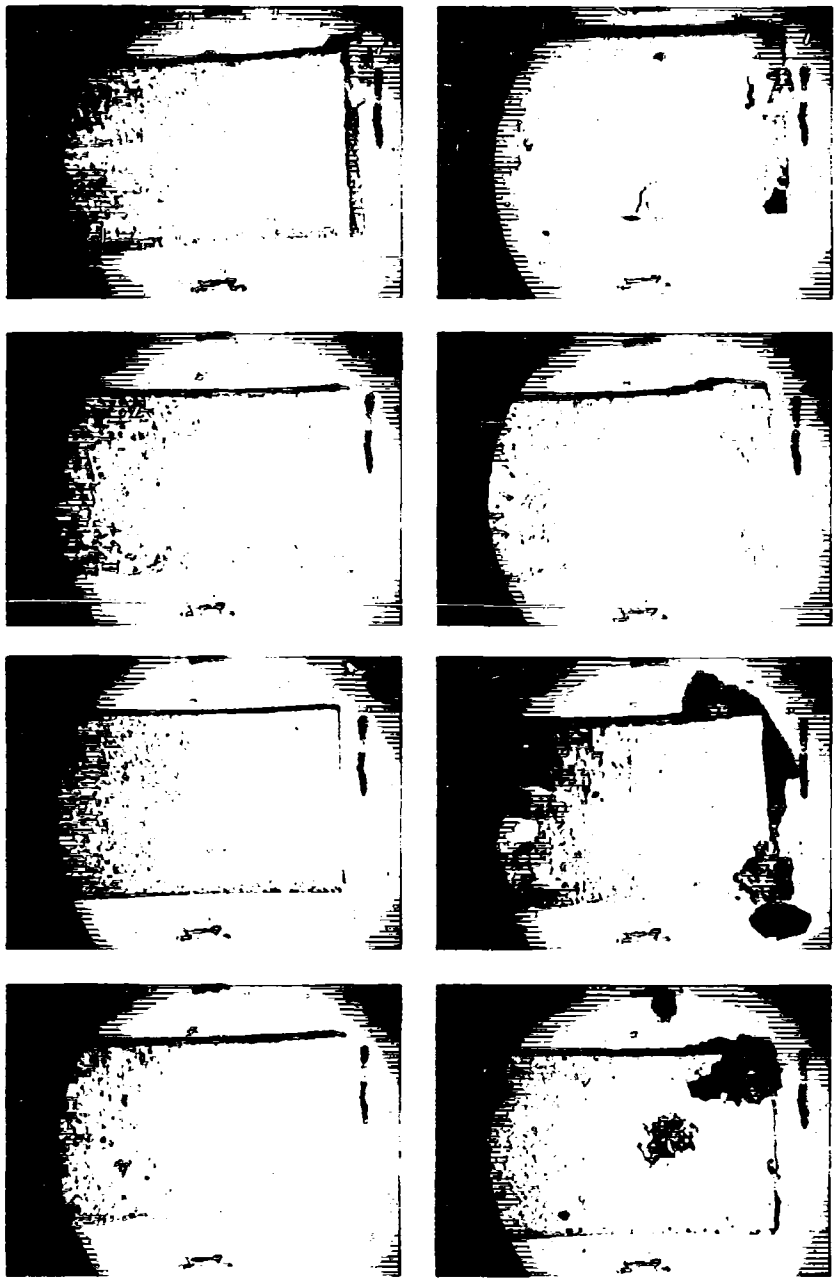


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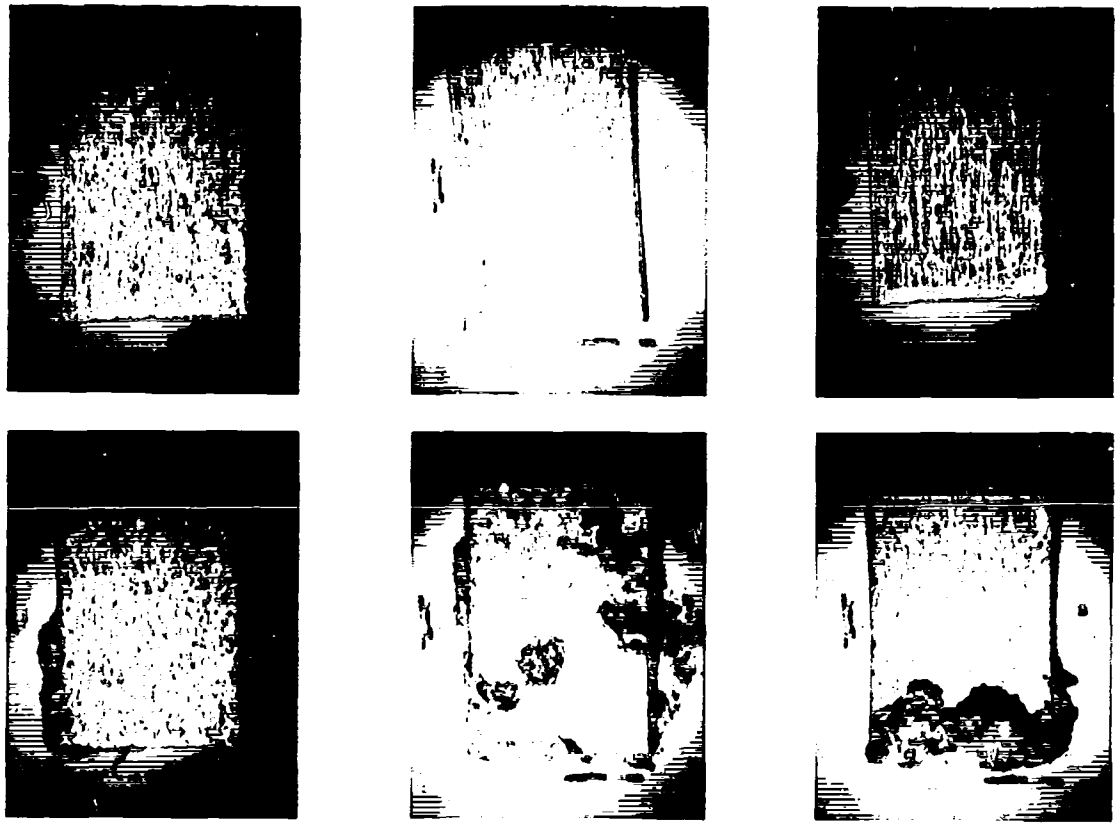
Figure 12. Corrosion of 7075 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 2 of 2)



LEGEND: FLASKS WERE INOCULATED WITH 5 ML OF MIXED CULTURE WHICH HAD BEEN GROWN 48 HOURS IN NBH-FUEL, HARVESTED BY CENTRIFUGATION AND WASHED 3 TIMES IN DISTILLED H_2O. AFTER INOCULATION THE FLASKS WERE PLACED AT 37°C FOR 97 DAYS ON A NEW BRUNSWICK SHAKER. THE TOP FIGURES REPRESENT UNINOCULATED CONTROL FLASKS CONTAINING SOLUTIONS, FROM LEFT TO RIGHT, OF 0, 0.02, 0.04, 0.06, 0.08, AND 1.2 GMS KNO_3 PER LITER AND THE LAST SOLUTION CONTAINING 1.0 GM OF $(NH_4)_2SO_4$ PER LITER. INOCULATED TEST FLASKS ARE REPRESENTED BY THE BOTTOM ROW OF FIGURES.

Figure 13. Corrosion of 7075 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 1 of 2)

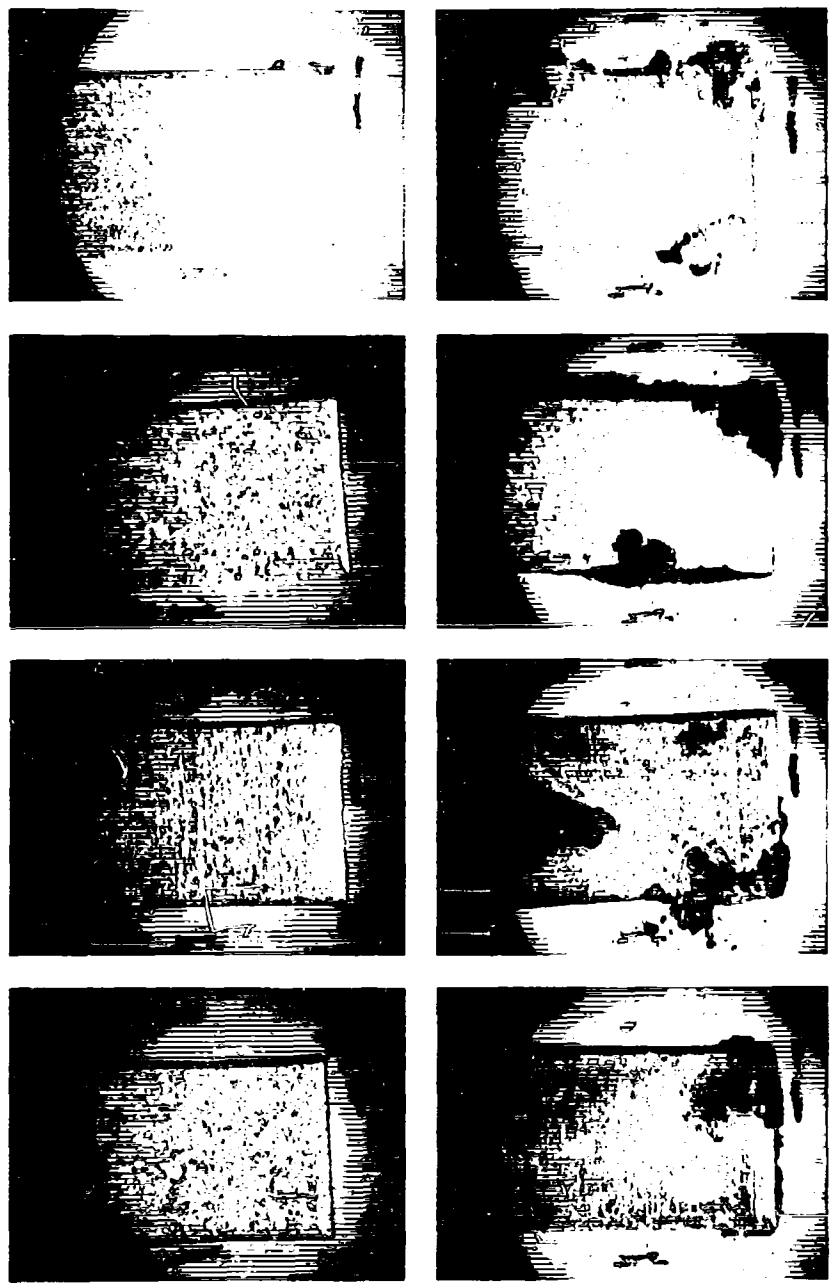


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Figure 13. Corrosion of 7075 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 2 of 2)

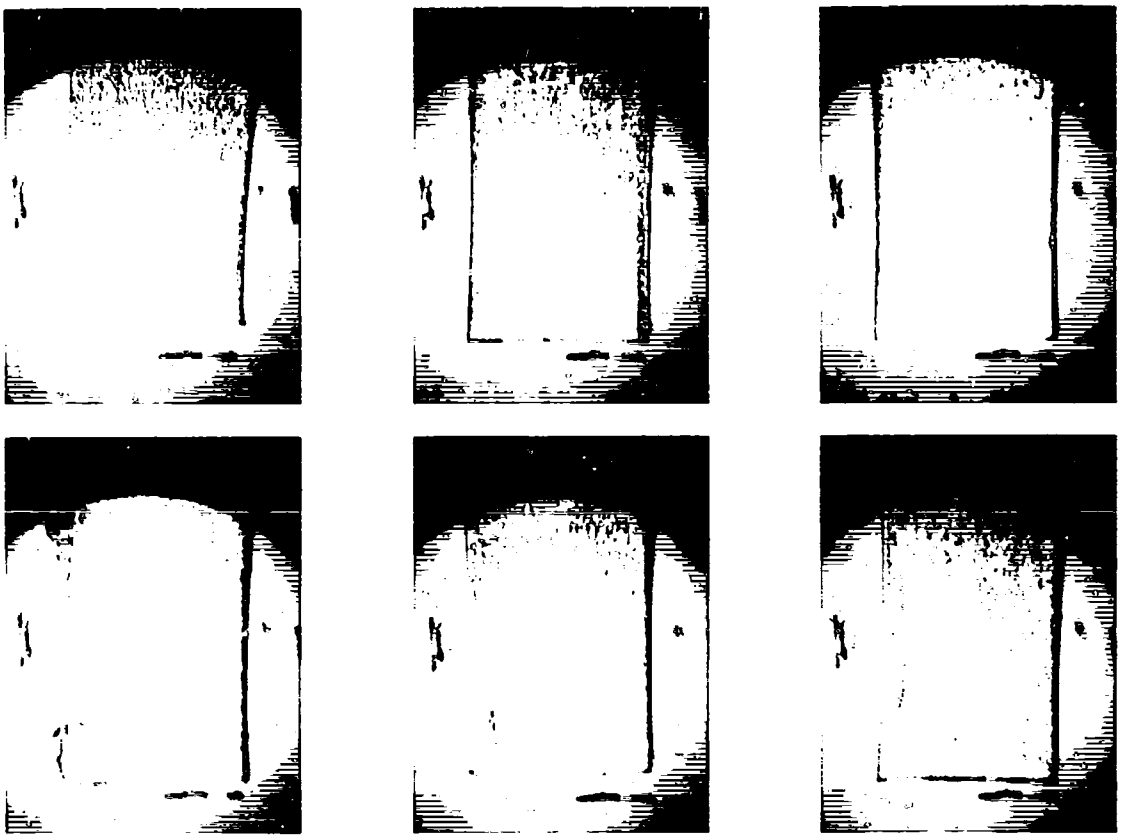

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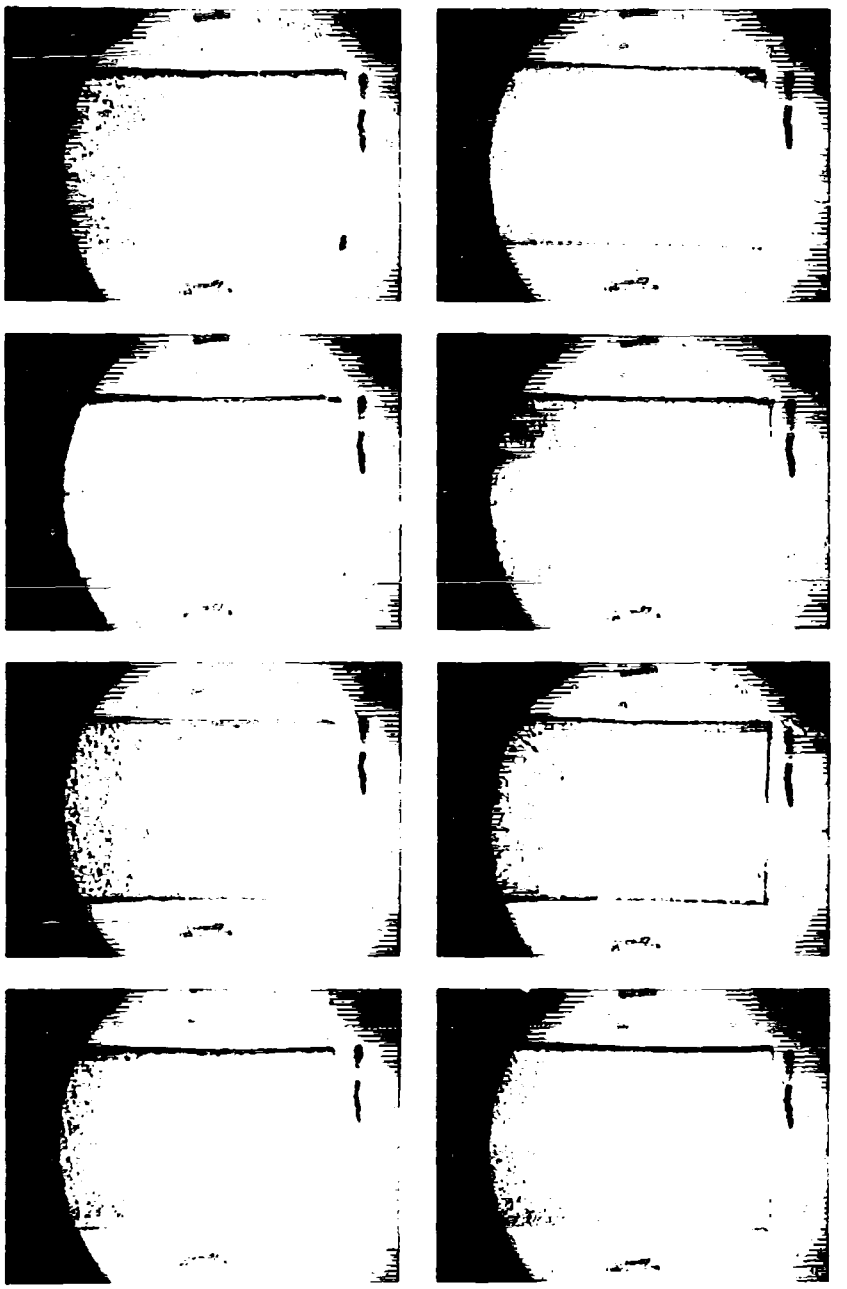
Figure 14. Corrosion of 2024 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 1 of 2)



LEGEND: FLASKS WERE INOCULATED WITH 5 ML OF MIXED CULTURE WHICH HAD BEEN GROWN 48 HOURS IN NBH-FUEL, HARVESTED BY CENTRIFUGATION AND WASHED 3 TIMES IN DISTILLED H_2O. AFTER INOCULATION THE FLASKS WERE PLACED AT 37°C FOR 20 DAYS ON A NEW BRUNSWICK SHAKER. THE TOP FIGURES REPRESENT UNINOCULATED CONTROL FLASKS CONTAINING SOLUTIONS, FROM LEFT TO RIGHT, OF 0, 0.02, 0.04, 0.06, 0.08, AND 1.2 gms KNO_3 PER LITER AND THE LAST SOLUTION CONTAINING 1.0 gm OF $(NH_4)_2SO_4$ PER LITER. INOCULATED TEST FLASKS ARE REPRESENTED BY THE BOTTOM ROW OF FIGURES.

Figure 14. Corrosion of 2024 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 2 of 2)

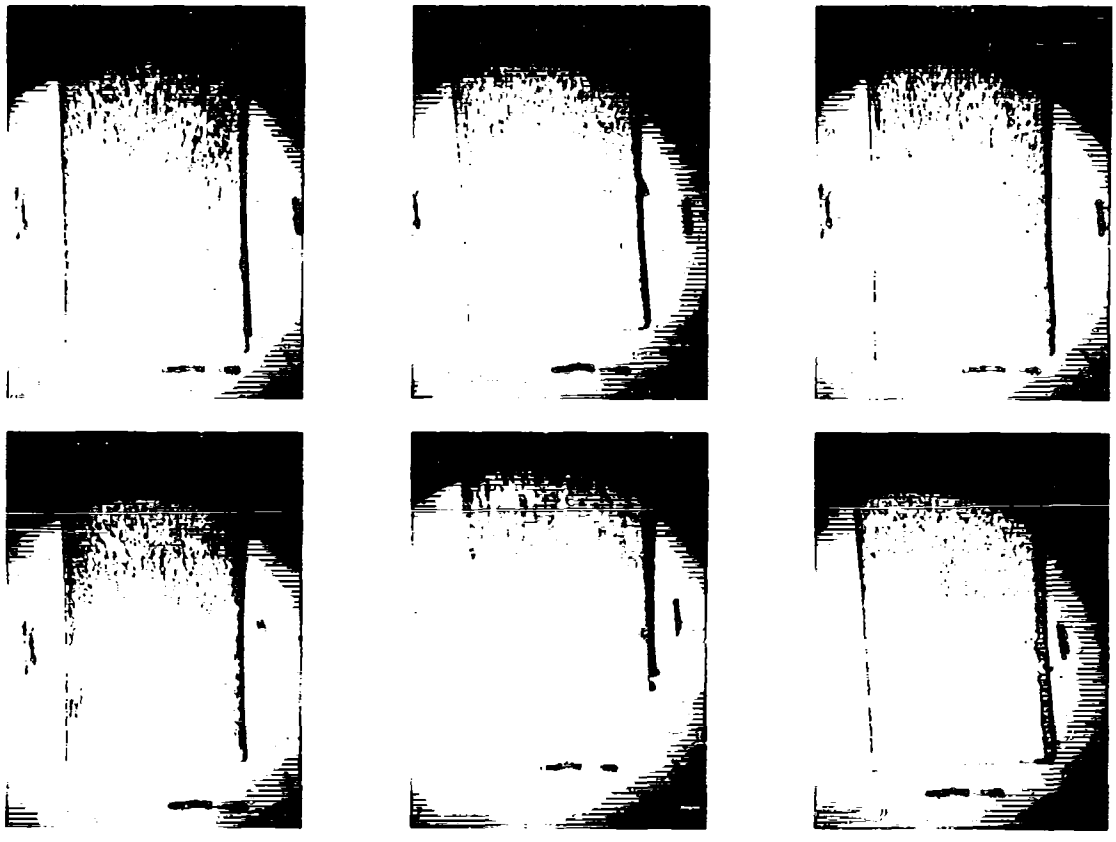


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Figure 15. Corrosion of 2024 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 1 of 2)

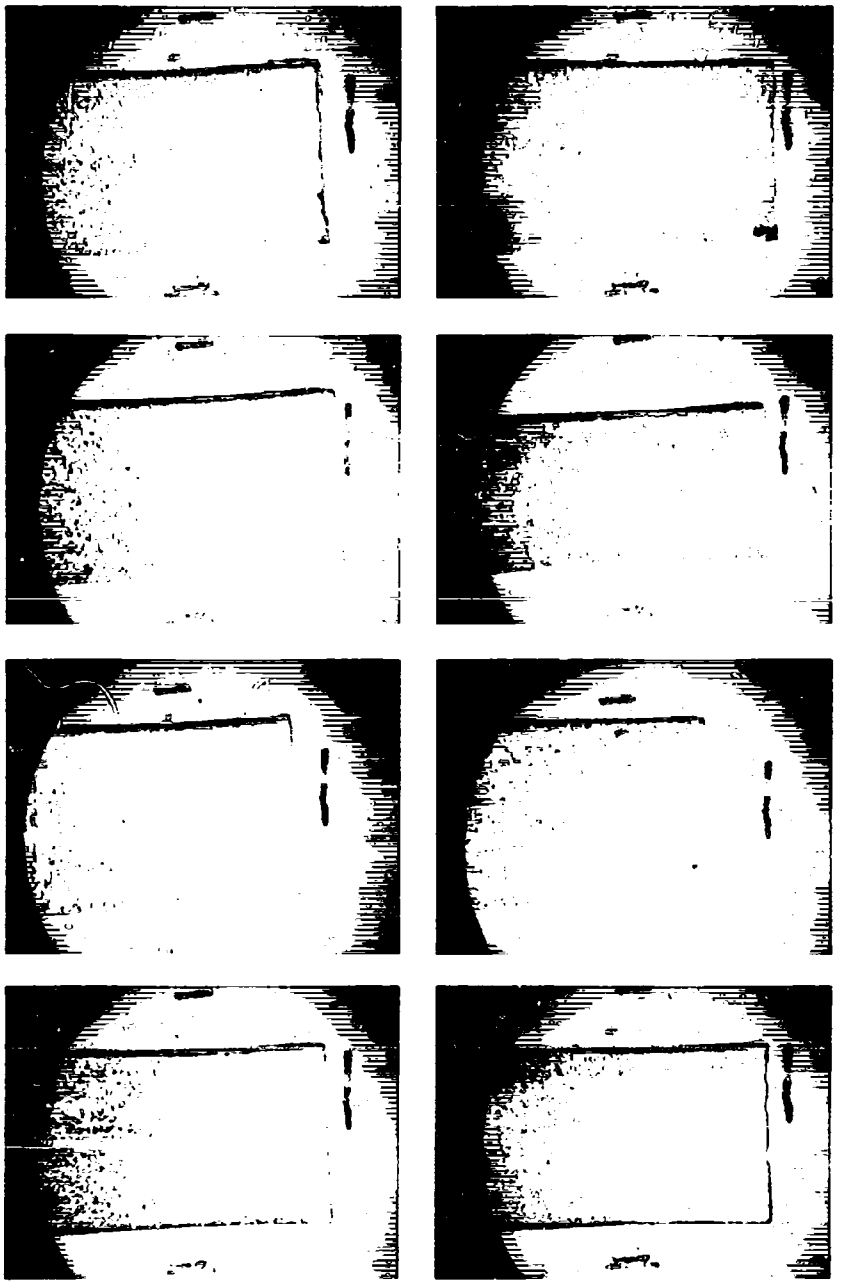

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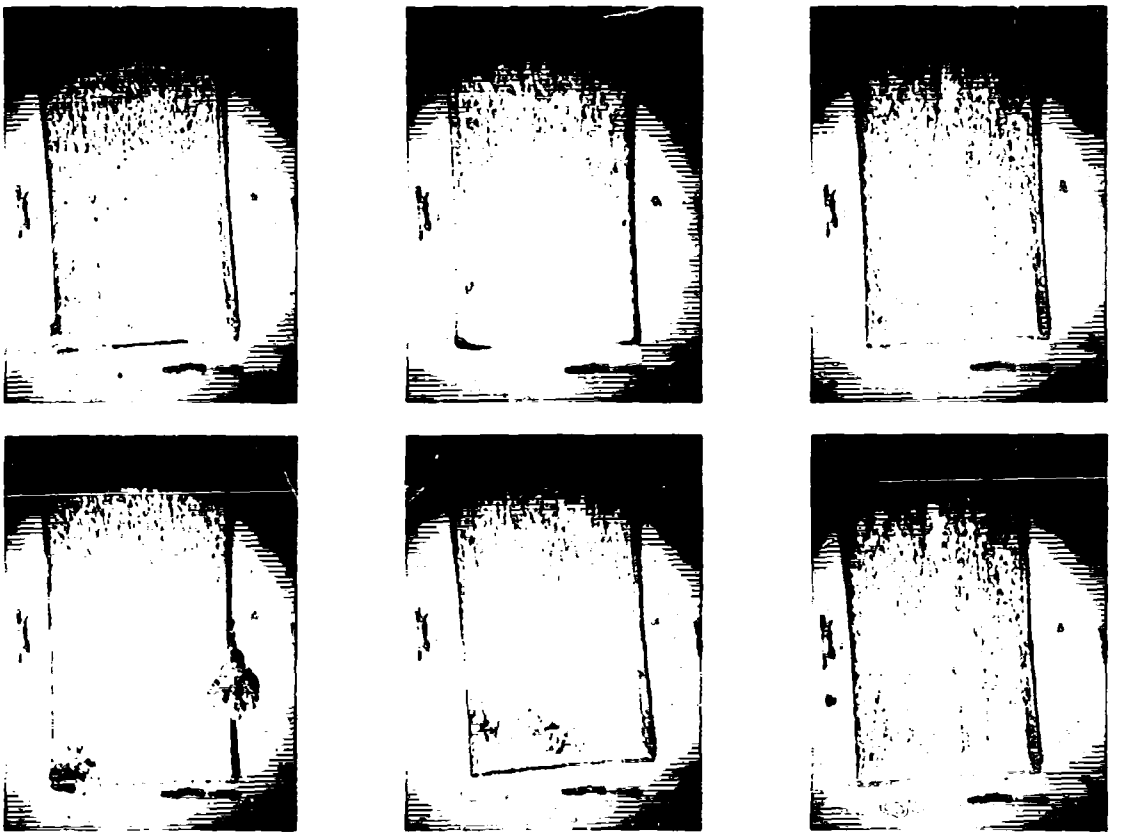
Figure 15. Corrosion of 2024 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 2 of 2)



LEGEND: FLASKS WERE INOCULATED WITH 5 ML OF MIXED CULTURE WHICH HAD BEEN GROWN 48 HOURS IN NBH-FUEL, HARVESTED BY CENTRIFUGATION AND WASHED 3 TIMES IN DISTILLED H_2O. AFTER INOCULATION THE FLASKS WERE PLACED AT 37°C FOR 60 DAYS ON A NEW BRUNSWICK SHAKER. THE TOP FIGURES REPRESENT UNINOCULATED CONTROL FLASKS CONTAINING SOLUTIONS, FROM LEFT TO RIGHT, OF 0, 0.02, 0.04, 0.06, 0.08, AND 1.2 GMS KNO_3 PER LITER AND THE LAST SOLUTION CONTAINING 1.0 GM OF $(NH_4)_2SO_4$ PER LITER. INOCULATED TEST FLASKS ARE REPRESENTED BY THE BOTTOM ROW OF FIGURES.

Figure 16. Corrosion of 2024 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 1 of 2)

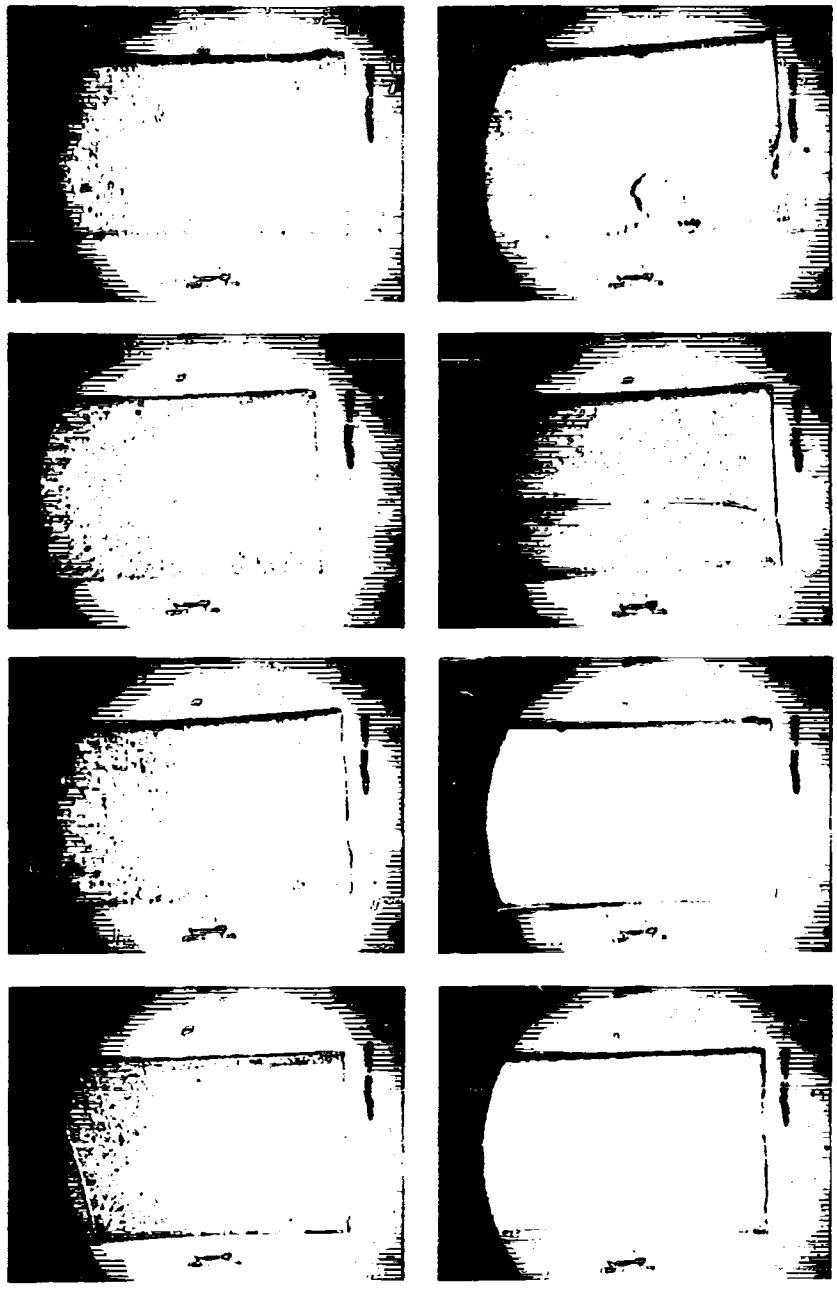


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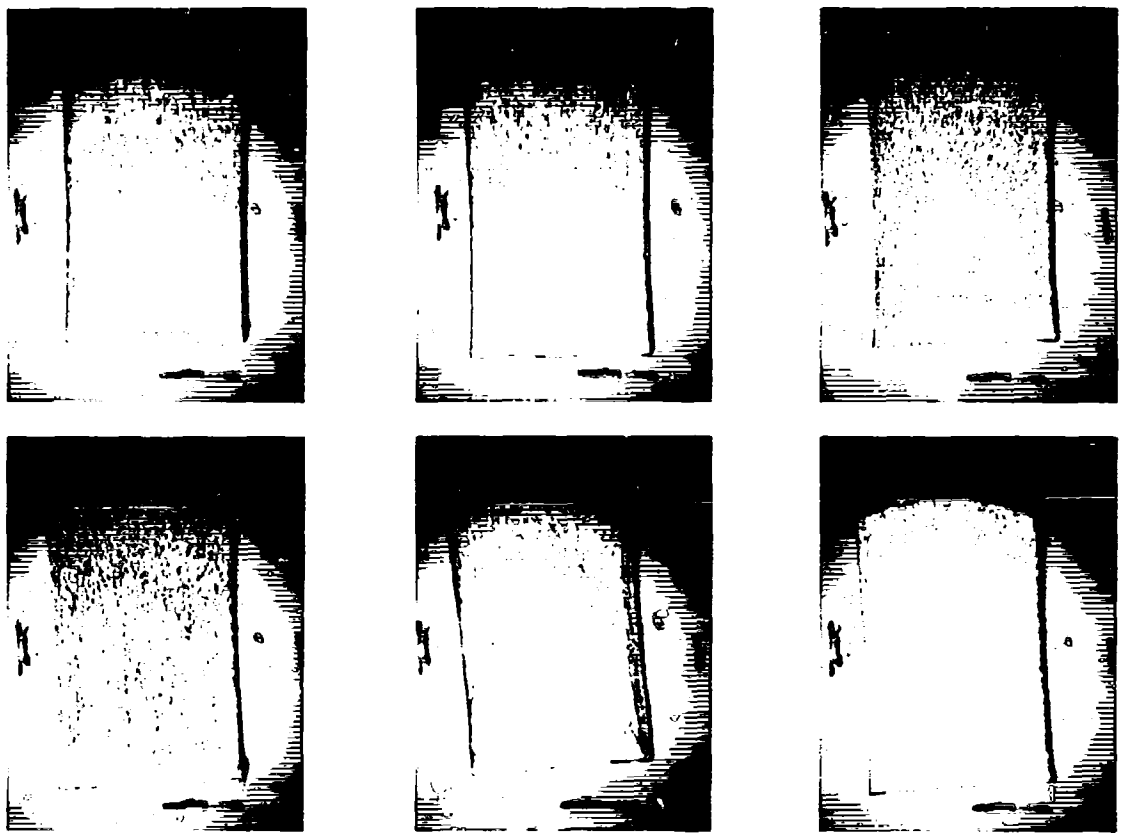
Figure 16. Corrosion of 2024 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 2 of 2)



LEGEND: FLASKS WERE INOCULATED WITH 5 ML OF MIXED CULTURE WHICH HAD BEEN GROWN 48 HOURS IN NBH-FUEL, HARVESTED BY CENTRIFUGATION AND WASHED 3 TIMES IN DISTILLED H_2O. AFTER INOCULATION THE FLASKS WERE PLACED AT 37°C FOR 80 DAYS ON A NEW BRUNSWICK SHAKER. THE TOP FIGURES REPRESENT UNINOCULATED CONTROL FLASKS CONTAINING SOLUTIONS, FROM LEFT TO RIGHT, OF 0, 0.02, 0.04, 0.06, 0.08, AND 1.2 GMS KNO_3 PER LITER AND THE LAST SOLUTION CONTAINING 1.0 GM OF $(NH_4)_2SO_4$ PER LITER. INOCULATED TEST FLASKS ARE REPRESENTED BY THE BOTTOM ROW OF FIGURES.

Figure 17. Corrosion of 2024 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 1 of 2)

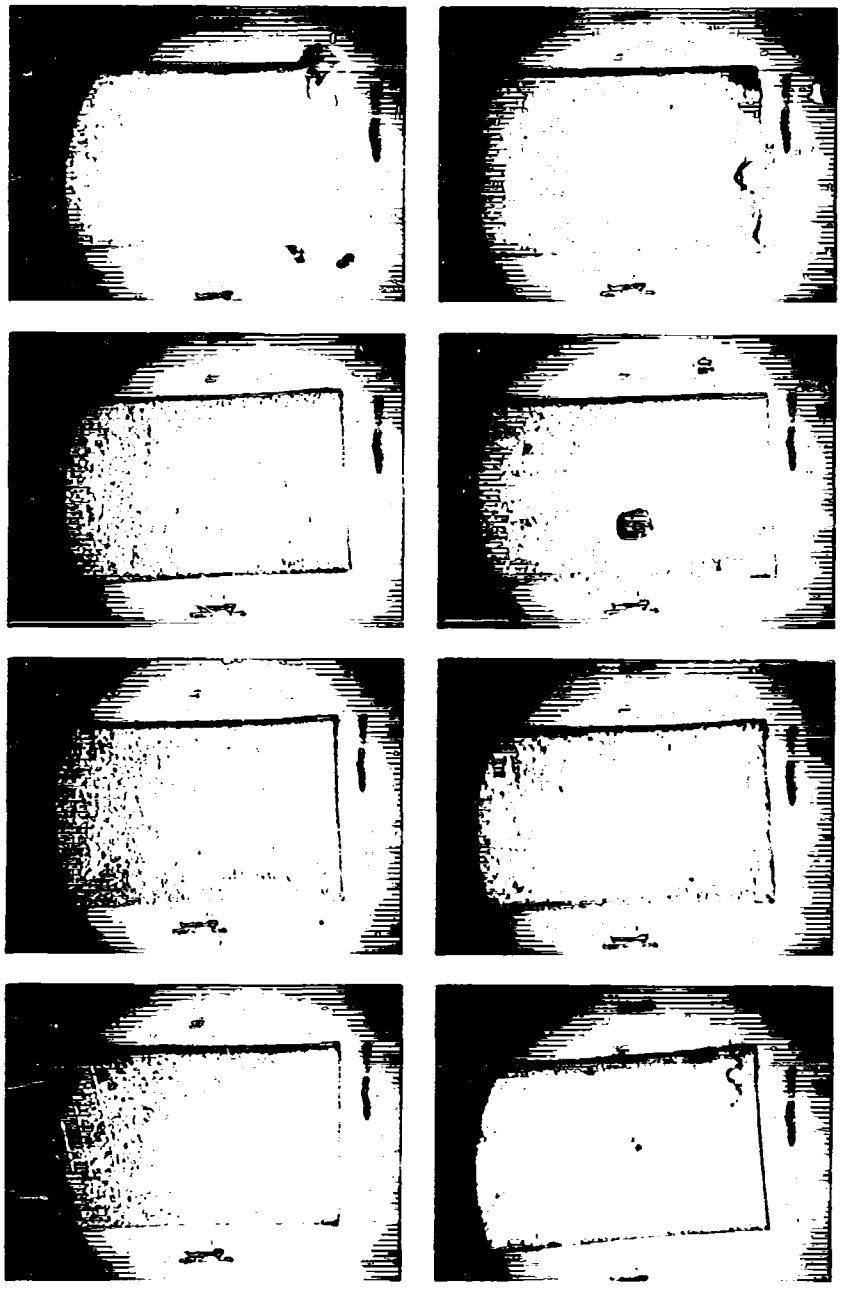


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Figure 17. Corrosion of 2024 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 2 of 2)

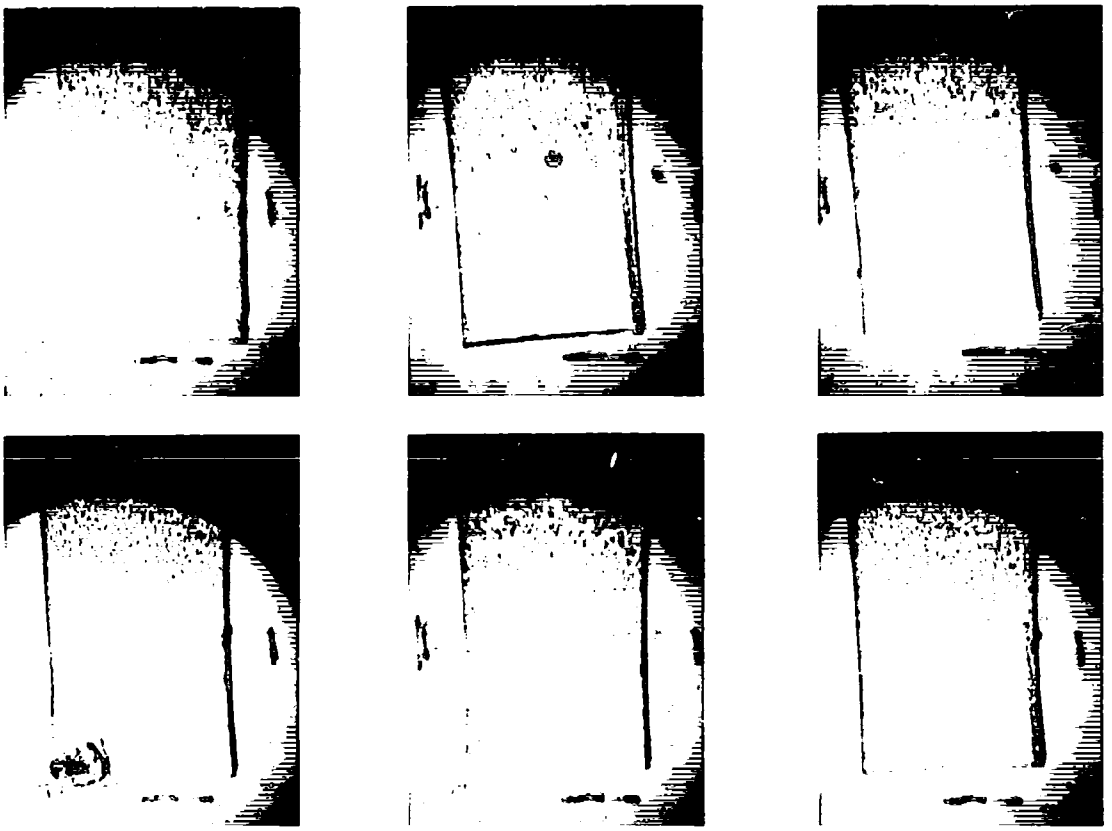

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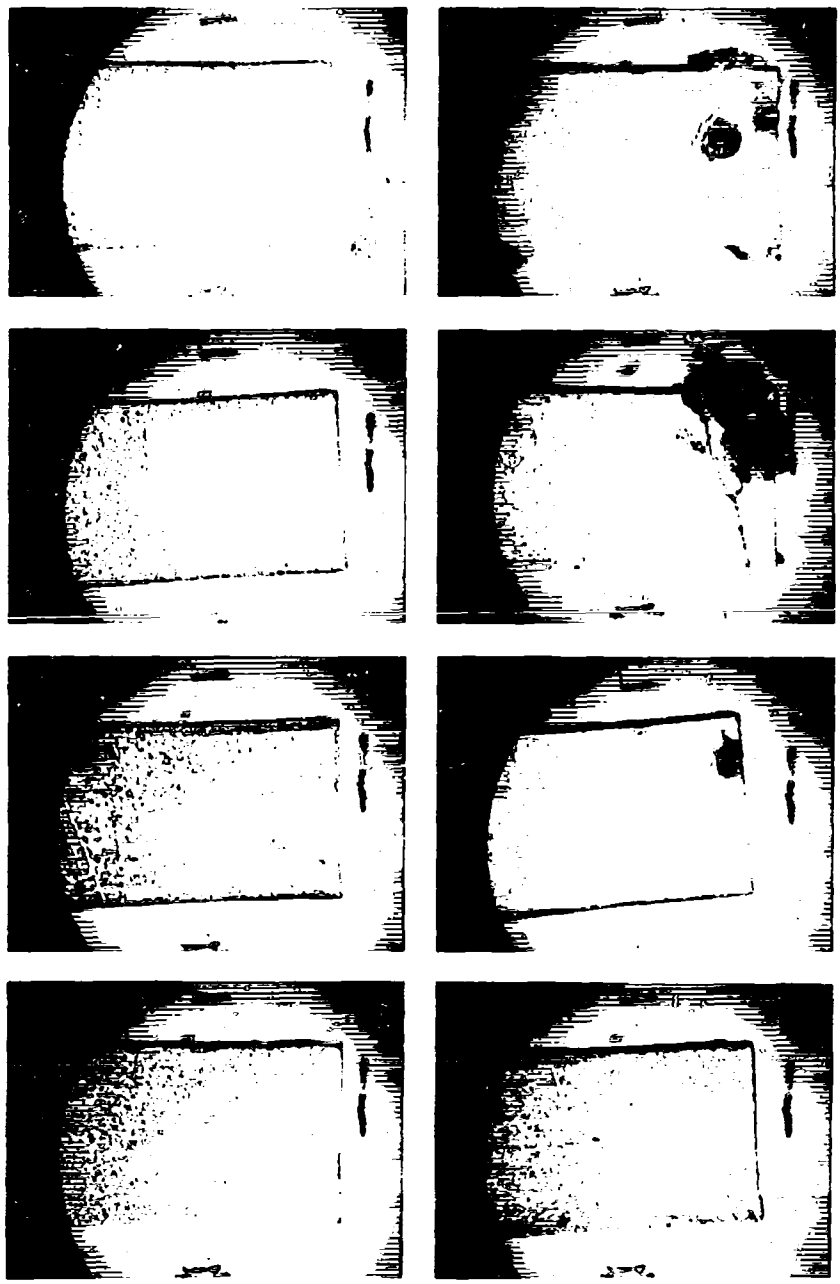
Figure 18. Corrosion of 2024 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 1 of 2)



LEGEND: FLASKS WERE INOCULATED WITH 5 ML OF MIXED CULTURE WHICH HAD BEEN GROWN 48 HOURS IN NBH-FUEL, HARVESTED BY CENTRIFUGATION AND WASHED 3 TIMES IN DISTILLED H_2O. AFTER INOCULATION THE FLASKS WERE PLACED AT 37°C FOR 97 DAYS ON A NEW BRUNSWICK SHAKER. THE TOP FIGURES REPRESENT UNINOCULATED CONTROL FLASKS CONTAINING SOLUTIONS, FROM LEFT TO RIGHT, OF 0, 0.02, 0.04, 0.06, 0.08, AND 1.2 gms KNO_3 PER LITER AND THE LAST SOLUTION CONTAINING 1.0 gm OF $(NH_4)_2SO_4$ PER LITER. INOCULATED TEST FLASKS ARE REPRESENTED BY THE BOTTOM ROW OF FIGURES.

Figure 18. Corrosion of 2024 Alloy by Mixed Culture in Media Containing Varying Concentrations of KNO_3 as the Only Nitrogen Source (Sheet 2 of 2)

In going from the top row of pictures to the bottom row in each figure a comparison is made of uninoculated and inoculated media; in going from left to right along any row the effect of increasing nitrate concentration on corrosion is observed; in going from Figure 9 to Figure 13 the occurrence of corrosion as a function of time at each nitrate concentration is shown for the alloy 7075. The latter comparison was made for alloy 2024 in Figures 14 through 18.

Figure 9 shows that in the absence of nitrate and in the absence of microbial growth, the corrosion of alloy 7075 took place within 20 days. The corrosion produced by microorganisms in this medium without added nitrate was appreciably greater than that observed in the sterile control. In a sterile control containing 0.02 g KNO_3 chemical corrosion was not apparent in 20 days but microbial growth caused corrosion in media containing as much as 0.06 g KNO_3 in this period of time. BH medium with $(NH_4)_2SO_4$ as nitrogen source did not cause corrosion in 20 days, but microorganism growing in this medium corroded alloy 7075 in this period.

The corrosion pattern observed in the first 20 days with alloy 7075 was indicative of that observed through a 97-day period. With the passage of time, corrosion was produced by microbial growth in media containing higher nitrate concentrations. In uninoculated controls, corrosion did not take place in media containing as little as 0.02 g KNO_3 per liter in 97 days, but extensive corrosion was observed in inoculated media with as much as 1.2 g KNO_3 per liter at the end of this period.

Nitrate inhibited the chemical corrosion of the 2024 alloy caused by the ions of the growth medium. But the corrosion of this alloy caused by microorganisms differed from that observed with the 7075 alloy. The microbial corrosion of the 2024 alloy appeared to take place only in the presence of large cell populations, while 7075 alloy corrosion was evident first at low nitrate and low cell concentration, and only much later at high nitrate and high cell concentration. Figure 14 shows corrosion of 2024 alloy in 20 days in inoculated basic medium without added nitrogen, and corrosion of this alloy is evident also in inoculated media which have ammonium sulfate substituted for nitrate. Figure 18 shows that microbial growth in media containing 0.02 g KNO_3 per liter does not corrode the 2024 alloy in 97 days, but the 2024 alloy was corroded by microbial growth in media containing 1.2 g KNO_3 per liter in this period of time.

Growth at high nitrate concentrations appeared to abruptly alter the physiological activity of microorganisms and permit the production of compounds not observed at lower nitrate concentrations. These compounds may account for the microbially produced corrosion of both the 2024 and 7075 alloys. High nitrate concentrations also appeared to invoke a new mechanism for the corrosion of aluminum, and this mechanism was effective in causing the corrosion of both the susceptible alloy (7075) and the more resistant alloy (2024).

This discontinuous rise in cell count with nitrate concentration is reflected in the production of colored material by organisms grown at the higher nitrate concentration. Table 2 shows the absorption at 420 mμ of the supernatantas obtained from cultures grown at different nitrate concentrations. The sharp increase in the concentration of absorbing material by cultures grown on 1.2 g KNO_3 per liter suggests that this high nitrate concentration may cause unique changes in the physiological character of the organisms. Organisms grown at this elevated nitrate concentration are ultimately more corrosive to both alloys tested than those grown at lower concentrations of nitrate.

These observations point up the ability of microbial flora to control the corrosive properties of the medium in which they grow and metabolize, and they represent a singular achievement in producing aluminum corrosion by microorganisms under controlled conditions.

3. Aluminum Corrosion Caused by Removal of Phosphate from Bushnell-Haas Medium During Growth of Fuel Microorganisms

Orthophosphate serves a dual function in the growth media of bacteria. It is a source of phosphate for cell growth, and it is a weak acid which buffers the medium near neutrality. Phosphorus, like nitrogen and carbon but unlike iron, magnesium, and calcium, constitutes the macrostructures of the bacterial cell. A study was therefore set up to investigate the ability of fuel-oxidizing bacteria to utilize phosphate and by so doing, make the medium in which growth takes place more corrosive toward aluminum.

Experiments were set up to determine the smallest concentration of phosphate that would permit the growth of fuel-oxidizing bacteria to population densities in the order of 10^7 to 10^9 cells per milliliter. Fuel-oxidizing organisms grew well in media that had low concentrations of nitrate as the only source of nitrogen. In testing the effect of phosphate on microbial growth, a medium was used with potassium nitrate at 0.06 grams per liter as the sole source of nitrogen. This nitrate concentration permits the demonstration of corrosion by ferric ion, or sodium chloride in about 24 hours. The medium was made to various phosphate concentrations ranging from 2.0 g per liter, which is approximately that of Bushnell-Haas medium, to 0.1 g per liter.

The observation has been made frequently that fuel-oxidizing organisms grown in BH medium bring this well-buffered medium to hydrogen ion concentrations around pH 4 to pH 5 at populations of 10^7 to 10^8 cells per ml. It was felt, therefore, that diminishing the phosphate concentration, while desirable for studies of microbial corrosion, would permit the hydrogen ion concentration of the medium to rapidly diminish and thereby prevent appreciable growth. It was of interest and of value to this study that the expected phenomenon did not occur. Thus, Table 3, even after seven days of growth the medium with 0.1 gm/l of K_2HPO_4 was at pH 7.3 but NBH medium had fallen in hydrogen ion concentration to pH 3.6.

TABLE 2.

OPTICAL DENSITY AT 420 mμ OF SUPERNATANTS FROM A MIXED CULTURE IN MEDIA CONTAINING VARYING AMOUNTS OF NITRATE

KNO_3 mM/liter	(Cells/ml)* x 10^{-8}	OD
0	0.5	0.243
0.02	1.4	0.416
0.04	2.7	0.476
0.06	2.8	0.650
0.08	2.3	0.730
1.2	10.0	1.9
NBH (ammonium sulfate)	4.0	0.236

* 3 days growth.

TABLE 3.

CHANGES IN GROWTH AND pH OF CULTURE 96 IN MEDIA WITH VARIOUS PHOSPHATE CONCENTRATIONS

Days	0.0gm/liter K_2HPO_4	pH	0.1gm/liter K_2HPO_4	pH	0.3	pH	0.5	pH	2.0 g/l K_2HPO_4	pH	NBH	pH
0	2.6×10^6	7.0	4.1×10^6	7.0	3.4×10^6	-	3.5×10^6	7.0	2×10^5	7.0	6.5×10^4	7.1
1	6.8×10^6	-	1.2×10^7	?	3.2×10^7	-	3.2×10^7	-	1.2×10^7	-	4×10^6	7.0
2	2.8×10^6	6.8	1.6×10^7	6.9	4×10^7	-	7.3×10^7	6.5	1.1×10^7	-	2.7×10^7	6.6
3	2.6×10^6	6.9	1×10^7	7.0	2.2×10^7	-	4.5×10^7	6.9	1.3×10^7	7.1	3×10^7	
7	*	6.8		7.3		7.1		7.0		7.1		3.6

* No counts made after 3 days.

The high cell population obtained in the medium with limited nitrate and phosphate concentrations revealed the possibility of testing the ability of organisms to fix phosphate within the cell and thus enhance the corrosivity of the medium in which they grow. The medium designed in this study also permits the growth of fuel-oxidizing organisms to relatively high population densities, while at the same time the medium appears to be minimally inhibitory to corrosion. Experiments were designed to test the ability of fuel organisms to cause corrosion in these media with various low concentrations of phosphate. The basic medium contained:

0.2 g/liter	$MgSO_4 \cdot 7H_2O$
0.2 g/liter	$CaCl_2$
0.5 g/liter	$FeCl_3$
0.06 g/liter	KNO_3

From this basic medium, four test media were prepared. The basic medium without additions and the basic medium with 0.1, 0.3, and 0.5 g K_2HPO_4 per liter added.

Each medium was dispensed into flasks and overlayed with JP-4 fuel. The corrosivity of the sterile medium was determined by adding alloy bars to each medium. Observations were made at 24 and 72 hours. Most corrosion occurred in media without phosphate (Figure 19) and the least corrosion occurred in media with 0.5 g K_2HPO_4 per liter (Figure 20).

Sterile media with aluminum coupons were inoculated with cells of the fuel isolate, culture 96. After 7 days incubation, no difference in corrosion was observed in the inoculated and control media without phosphate added. Figure 19 shows that in the absence of phosphate, this medium causes corrosion to about the same extent in the presence as in the absence of microorganisms. Similar results were obtained with media containing 0.3 g/liter of K_2HPO_4, but after 7 days growth, corrosion was seen in inoculated media containing 0.5 g/liter of K_2HPO_4, but corrosion was essentially absent from aluminum coupons in the uninoculated control, Figure 20.

The chemical mechanism responsible for phosphate inhibition of aluminum corrosion is unclear, but the formation of complex compounds of aluminum and phosphate, or phosphate and some unknown ion catalizing corrosion is a possibility. The inhibition of corrosion by nitrate, however, is probably not accomplished by this mechanism. Both nitrate and phosphate are taken up by the bacteria cell and this activity has been shown to be accompanied by increases in the corrosivity of the growth medium.

4. Aluminum Corrosion in Proteinaceous Media

In this study, the possibility has been emphasized that bacterial metabolism may effect large changes in the electrochemical properties of very confined areas by establishing microcenters of galvanic activity. Such centers would exist where microbial colonies or materials were affixed to

LEGEND: FROM LEFT TO RIGHT, 2024 AND 7075 ALLOYS ARE REPRESENTED. CONTROL STRIPS PRESENTED AT THE TOP WERE IMMERSED IN MEDIA CONTAINING NO PHOSPHATE AND NO BACTERIA. THE BOTTOM FIGURES REPRESENT TEST STRIPS WHICH WERE IMMERSED IN MEDIA OF THE SAME COMPOSITION, INOCULATED WITH 5 MLS OF BACTERIAL CULTURE. THE FIGURES REPRESENT THE ALLOYS AFTER 7 DAYS INCUBATION AT 30°C.

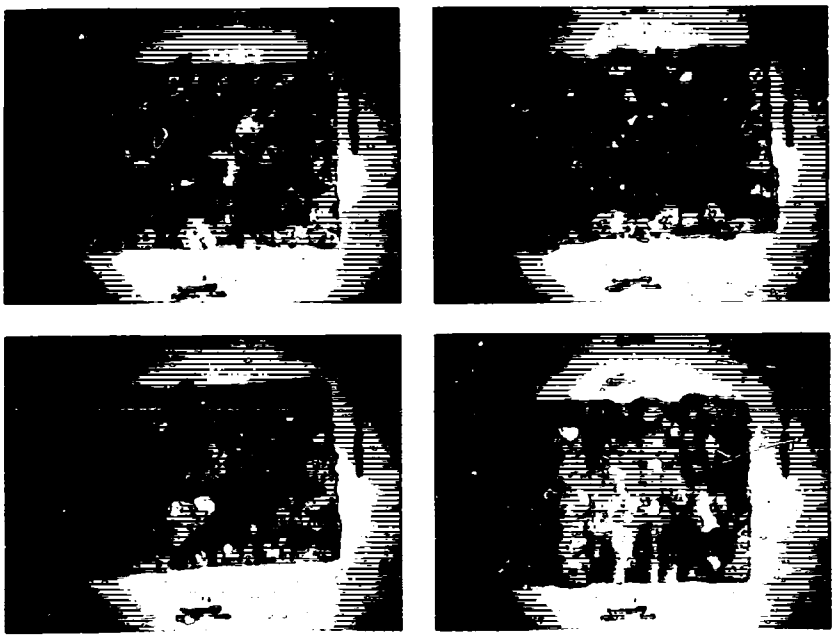


Figure 19. The Corrosion of Aluminum Alloys in Culture 96 in Medium Containing no Phosphate

LEGEND: FROM LEFT TO RIGHT, 2024 AND 7075 ALLOYS ARE REPRESENTED. CONTROL STRIPS PRESENTED AT THE TOP WERE IMMERSED IN MEDIA CONTAINING NO PHOSPHATE AND NO BACTERIA. THE BOTTOM FIGURES REPRESENT TEST STRIPS WHICH WERE IMMERSED IN MEDIA OF THE SAME COMPOSITION, INOCULATED WITH 5 MLS OF BACTERIAL CULTURE. THE FIGURES REPRESENT THE ALLOYS AFTER 7 DAYS INCUBATION AT 30°C.

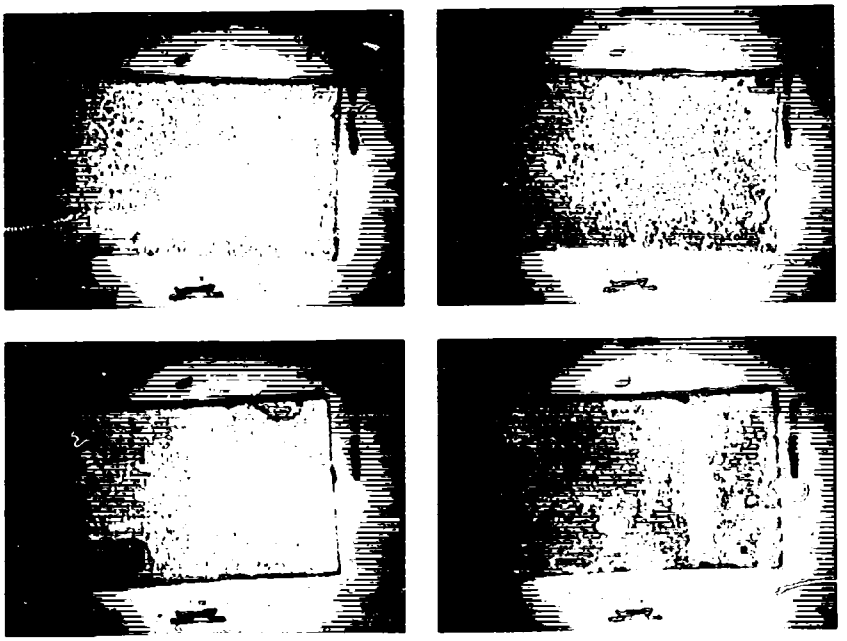


Figure 20. The Corrosion of Aluminum Alloys in Culture 96 in Medium Containing 0.5 Grams of Phosphate Per Liter

aluminum surfaces, whereas galvanic activity could derive from the resulting restriction of diffusion either of oxygen or of metabolic products.

Should the restriction of oxygen diffusion be of importance to the production of corrosion, then the bacterial mass causing corrosion need not be viable. This hypothesis was tested as described in the following: The major chemical constituent of the bacterial cell is protein and following death and cell lysis this protein is liberated into the growth medium. Experiments were performed to determine the effect of protein hydrolysate on the corrosion of aluminum both in the presence and in the absence of viable cells.

Solutions, 5% in casein hydrolysate and prepared in distilled water, were adjusted to pH 7 with NaOH. Coupons of aluminum alloys 7075 and 2024 were submerged in these sterile protein solutions. The protein solutions with coupons and a jet-fuel overlay were incubated for 14 days. During this interval, a progressive deposition of protein on the aluminum bars occurred even though the entire system was subjected to continuous and virgorous agitation. After 14 days, the coupons were thoroughly examined. The local deposits of denatured protein material were removed revealing an aluminum surface which suffered a severe corrosion, but corrosion definitely confined to areas of protein deposit.

This observation was suggestive evidence that the products of microbial lysis, amino acids, peptides, and proteins can adhere to alloys 2024 and 7075 and that these products are capable of initiating corrosion of aluminum fuel tanks. Also, it has been reported in the literature that bacteria growing on fuel produce amino acids and proteins which are excreted into their growth medium. These reports and observations necessitate a consideration of aluminum corrosion in relation to classes of compounds other than fatty acids and hydrocarbons, and they suggest the existence of a mechanism of microbial corrosion which depends on the synthetic activity of microorganisms as well as their ability to alter the mineral content of a growth medium.

The presence of organisms in fuel-water bottoms, which are incapable of oxidizing fuel, suggests that these environments contain a variety of organic contaminants that may be used as sources of carbon. Such carbon sources may derive from lysed organisms, from organic matter, from soil run off, or from material taken into aircraft breathers. This material may cause aluminum corrosion directly or may stimulate the production of corrosive compounds by organisms present in water bottoms which do oxidize fuels.

To test these hypotheses an experiment was set up using 5% casein hydrolysate in H_2O, pH 7.0, and alloys 2024 and 7075 were submerged in this sterile medium. After 19 days in this medium, corrosion had occurred on both alloys. This experiment was repeated and expanded to include casein hydrolysate alone and with BH-Salts or KNO_3.

A 5% solution of casein was prepared in distilled H_2O and pH was adjusted to 7.0. This solution was dispensed into Erlenmeyer flasks. The casein hydrolysate at 1.2×10^{-2} M KNO_3, and casein hydrolysate plus BH-salts were prepared.

All media were overlayed with JP-4 fuel. As an inoculum 5 ml of culture 101 containing 1.9×10^9 organisms per ml were added to 5 test flasks of each medium. Flasks for each medium were inoculated and sterile controls were maintained. Observations were made at 4, 7, and 15 days.

Table 4 shows the hydrogen ion concentration and viable cell count after 48 hours growth in these media. The pH rose slightly and the yield of organisms in casein, hydrolysate casein, hydrolysate plus KNO_3, and casein hydrolysate plus BH-salts did not differ significantly; yet at the end of 15 days incubation, the corrosion produced by organisms grown in these media did differ significantly.

As shown in Figure 21, corrosion is produced by microorganisms in casein alone on the 2024 and 7075 alloy. As observed previously, the 7075 alloy is appreciably more affected by microbial corrosion than 2024. The effect of nitrate on the corrosion of aluminum alloys by bacteria grown in casein hydrolysate is shown in Figure 22.

The microbial corrosion is more severe in the presence of nitrate than in the absence of this compound. These results suggest that the corrosion produced by growth in casein hydrolysate was produced by a mechanism differing from that operative in an inorganic medium with a fuel overlay. It should be emphasized that the concentration of nitrate used in this casein medium was sufficient to prevent the occurrence of corrosion in 97 days in a sterile medium containing potentially corrosive ions. The effect of casein hydrolysate plus the salts of the Bushnell-Haas medium is shown in Figure 23. The greatest corrosion was observed in this medium. The extent of this corrosion suggests that some property or constituent of casein destroys the ability of the nitrate to inhibit corrosion caused both by growth on casein and by the corrosive cations of the BH-medium.

In the past, the theory was tested to determine whether microbial corrosion of aluminum takes place because microorganisms utilize naturally occurring corrosion inhibitors. These observations with casein hydrolysate suggest a second mechanism of microbial corrosion in which the organisms actually produce materials that cause corrosion. Future work will be concerned with the effect of products from lysed organisms on the corrosion of aluminum.

The investigation of conditions necessary and sufficient for the production and inhibition of microbial corrosion of aluminum alloys will be continued, and special consideration will be given to a statistical analysis of the incidence of corrosion in relation to the ionic content of different growth media.

TABLE 4.

GROWTH AND pH CHANGES OF CULTURE 101 IN THREE CASEIN HYDROLYSATE MEDIA

Day	5% Casein Hydrolysate		5% Casein Hydrolysate + 1.2×10^{-2} M KNO_3		5% Casein Hydrolysate + BH salts	
		pH		pH		pH
0	2.9×10^{6}	7.2	2.5×10^{7}	7.2	1.7×10^{7}	7.2
1	5.7×10^{8}	7.3	7.6×10^{8}	7.4	6.3×10^{8}	7.3
2	9×10^{8}	7.5	3.7 c 10^{8}	7.4	1×10^{9}	7.4

LEGEND: CONTROL BARS OF 2024 AND 7075 ALLOY ARE REPRESENTED BY THE TOP FIGURES. TEST FIGURES ARE PRESENTED AT THE BOTTOM; THEY WERE IMMERSED IN MEDIA THAT CONTAINED CULTURE 101. THE INOCULUM WAS GROWN IN FUEL HARVESTED BY CENTRIFUGATION AND WASHED 3 TIMES IN DISTILLED H_2O. TIME OF TEST--15 DAYS.

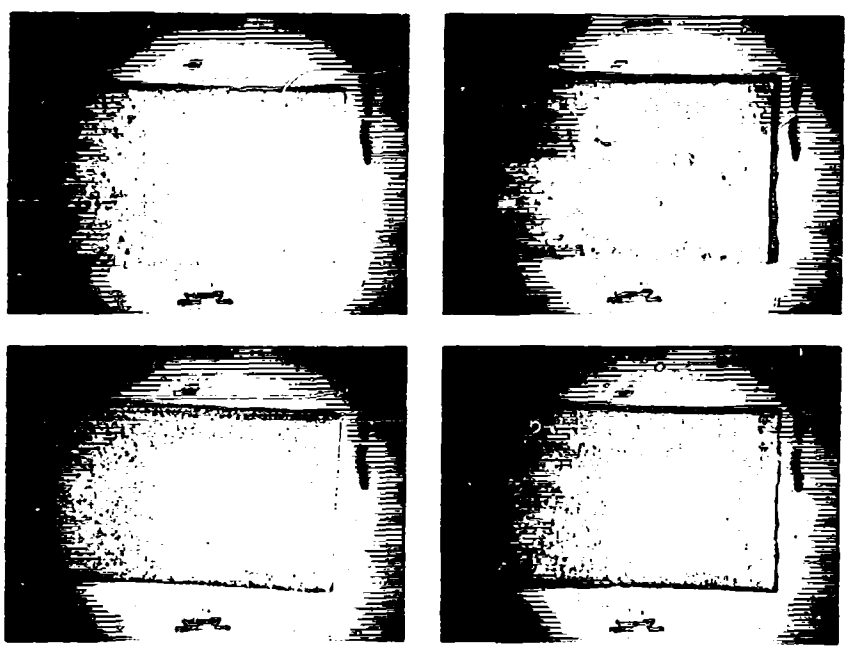


Figure 21. Corrosion of Aluminum Alloys in 5% Casein Hydrolysate Medium Containing Culture 101

LEGEND: UNINOCULATED CONTROLS ARE PRESENTED AT THE TOP. THE 2024 IS PRESENTED AT THE LEFT AND THE 7075 AT THE RIGHT. TEST ALLOYS REPRESENTED AT THE BOTTOM WERE IMMERSED IN MEDIA CONTAINING FUEL-GROWN CELLS WHICH HAD BEEN WASHED IN DISTILLED WATER 3 TIMES. TIME OF TEST--15 DAYS.

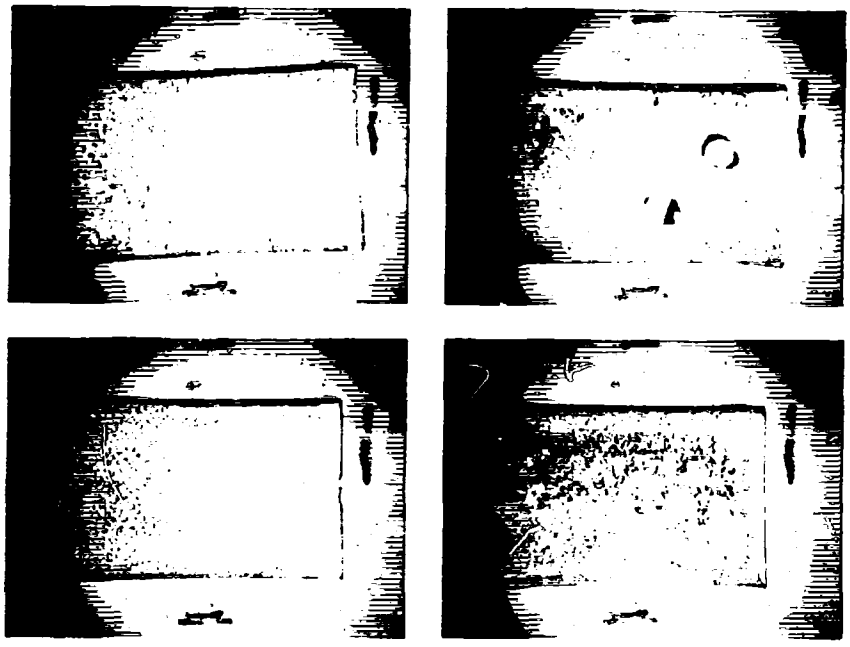


Figure 22. The Effect of Nitrate on the Corrosion of Aluminum Alloys by Culture 101 in 5% Casein Hydrolysate

LEGEND: UNINOCULATED CONTROLS ARE PRESENTED AT THE TOP. THE 2024 IS PRESENTED AT THE LEFT AND THE 7075 AT THE RIGHT. TEST ALLOYS REPRESENTED AT THE BOTTOM WERE IMMERSED IN MEDIUM CONTAINING FUEL-GROWN CELLS WHICH HAD BEEN WASHED IN DISTILLED WATER 3 TIMES. TIME OF TEST--15 DAYS.

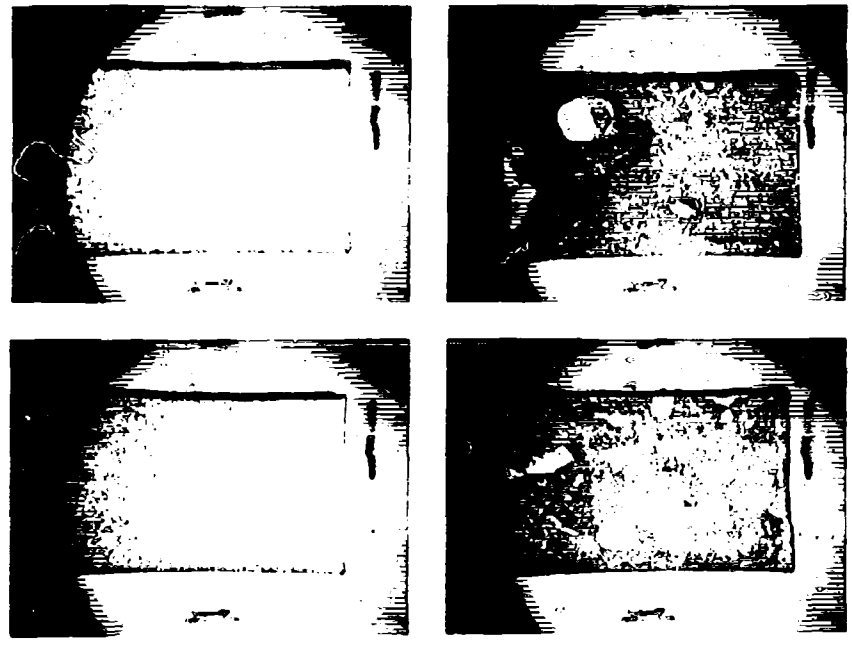
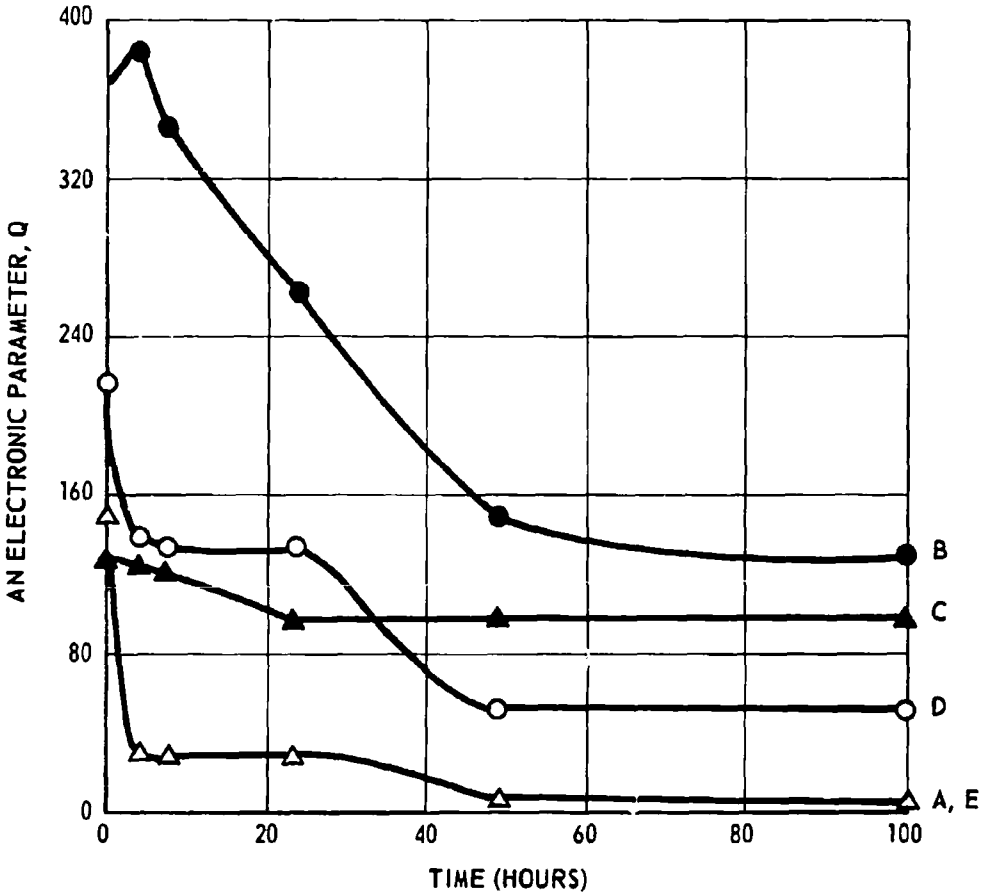


Figure 23. The Effect of BH-Salts on the Corrosion of Aluminum Alloys by Culture 101 in Casein Hydrolysate

In the present study, the occurrence of aluminum corrosion was followed both by photography and by the use of pulse polarization analysis. This electronic analytical method was able to reveal the tendency of a medium to cause corrosion long before the corrosion became visually detectable. A comparison of the curves in Figure 24 with the corroded alloy shown in Figure 25 indicated that changes in the pulse polarization parameter, Q, took place almost immediately upon placing the alloy in solutions of corrosive ions, although corrosion was not visible for about 24 hours. In a medium with nitrate at inhibitory concentrations, the Q value changed little at zero time and changed slowly with the passage of time. The corrosion shown in Figure 25 had taken place in a 5-day period. Results obtained by pulse polarization analysis indicate the possibility of following the time course of corrosion as well as characterize the type of corrosive agent acting through the pulse polarization response of the alloy under analysis.



A: 8×10^{-3} M $CaCl_2$;

B: 8×10^{-3} M $CaCl_2$; 1.2×10^{-2} M KNO_3

C: 8×10^{-3} M $CaCl_2$; 1.2×10^{-3} M KNO_3

D: 8×10^{-3} M $CaCl_2$; 1.2×10^{-4} M KNO_3

E: 8×10^{-3} M $CaCl_2$; 1.2×10^{-5} M KNO_3

Figure 24. Changes in Pulse Polarization Characteristics of Aluminum 2024 with Time and Corrosion Inhibitor Concentration

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ALL SOLUTIONS WERE AT 8 X 10^{-3} M $CaCl_2$, AND SOLUTIONS A, B, C, D, AND E ARE RESPECTIVELY AT 0, 1.2 X 10^{-2}, 1.2 X 10^{-3}, 1.2 X 10^{-4} AND 1.2 X 10^{-5} MOLAR KNO_3. THE PICTURE WAS TAKEN AFTER 5 DAYS INCUBATION IN THESE SOLUTIONS. THE CORROSION OF THESE COUPONS WAS FOLLOWED BY PULSE POLARIZATION ANALYSIS.

Figure 25. The Corrosion of Aluminum Alloy 2024 in $CaCl_2$ Solutions at Various KNO_3 Concentrations

B. Jet Fuel Contamination by Metabolic Products and the Metabolism of Jet Fuel Isolates

Microorganisms affect jet fuel systems by the chemical transformations brought about by means of enzymes. Because microbes use these biologically produced catalysts to cause chemical changes, the amount of chemical change produced by them does not bear a simple relationship to their size. A single cell may produce manyfold its weight in acid in unit time.

Microbial activity in a fuel system may be visualized as resulting in two forms of contamination. The hydrocarbons of fuel are transformed by microorganisms into the components of the microbial cell body, and into products like organic acids and esters which change the composition and surface activity of the fuel system.

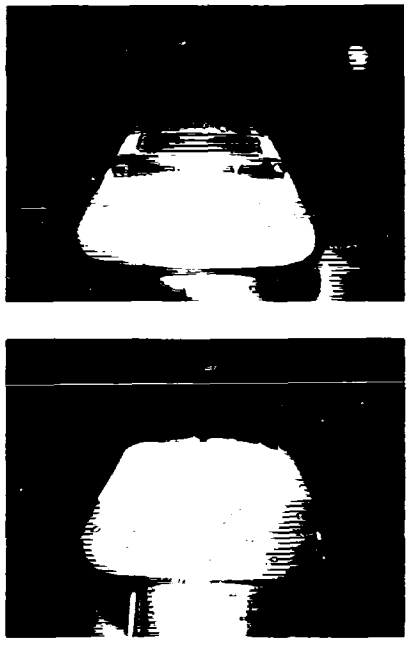
The problems associated with microbial growth in fuel systems may be approached on the level of the enzyme or on the level of the cell. The chemical changes brought about by the enzymes of microorganisms, such as the emulsion formation, may be prevented by inhibiting the activities of enzymes preventing their formation.

On the basis of these considerations, it was felt that a study of the metabolism of fuel isolate was an indispensable adjunct to understanding chemical changes in fuel and in fuel contaminants brought about by microorganisms.

1. Jet Fuel Phase Penetration and Emulsion Formation by Microorganisms

The water bottom in a fuel-water system is the most significant locus of microbial contamination. It is highly certain that microbial products, such as emulsions that contaminate fuel systems, are formed at a water-fuel interface and that they are discharged into the fuel phase under certain conditions of pH, agitation, and temperature. It is believed also that organisms which contribute most to fuel contamination are those capable of oxidizing hydrocarbons. This phase of the program was devoted to a study of the enzymes and metabolic pathways used in fuel degradation and contaminant production.

The ability of microorganisms to penetrate a fuel layer was shown by Figure 26. That this was a characteristic which varied from organism to organism was apparent. In this figure cells of the fuel isolate, culture 96, entered the fuel phase in a short period of time while cells of culture 101, also a fuel isolate, entered the fuel phase only after long growth periods and then only to a small extent. Culture 96 yielded cells that also distributed themselves homogeneously in the aqueous phase of a fuel-water system, but with continued growth the organisms concentrated at the fuel-water interface. These organisms appeared to form an "emulsion", and with old cultures this bacterial mass progressively penetrated the fuel phase.



LEGEND: THE FERNBACH FLASKS CONTAINED 1 LITER OF NBH MEDIUM AND 200 MLS OF JP-4 FUEL. THE FLASKS WERE INOCULATED WITH 100 MLS OF CULTURE 96 AND 101 RESPECTIVELY. THE TOP FLASK OF 101 AND THE BOTTOM FLASK OF 96 ARE REPRESENTED AFTER 48 HOURS GROWTH.

Figure 26. A Comparison of the Ability of Cultures 96 and 101 to Penetrate a Jet Fuel Layer

Studies were performed to characterize the water phase and interface organisms. The latter organisms could not be centrifuged down indicating that their surface properties were altered or that for some reason their specific gravity had become less than water with continued growth on fuel.

The viability and respiration of top and bottom cells of culture 96 were compared. Difficulties were encountered in making microscopic counts of the top cells because of their tendency to aggregate at the top of an aqueous medium. Nevertheless, the cells could be dispersed by vigorous shaking with water and counting was possible with samples removed immediately after shaking.

On the basis of cell counts, the percentage of viable cells in the top and the bottom layers was essentially the same. As shown in Table 5, the floating top layer cells were almost as metabolically active as their bottom counterpart.

Attempts were made to elucidate the metabolic mechanism of emulsion formation by studying the growth of fuel isolates on purified hydrocarbons. It was felt that growth on certain hydrocarbons would lead to emulsion formation more quickly than growth on others. Table 6 shows the populations of cells obtained after various time intervals on purified materials and the final pH of the growth medium.

The hydrocarbons, 1-tetradecene and 1-decene, were the superior sources of carbon with respect to growth, but only on 1-tetradecene and on jet fuel was "emulsion" formed.

The substance responsible for the diminished specific gravity of the top cells was a material associated with hydrocarbon oxidation; this phenomenon of flotation was not observed with cells grown on TGY medium. The material synthesized by these cells, which causes them to float, is a potential fuel contaminant, and it may have the ability to affix to debris in water and bring it to a fuel-water interface, thereby enhancing the problem of contamination.

To study the chemical and physical properties of the "emulsion" produced by the floating cells, the aqueous phase was separated from the fuel phase in a separatory funnel. The protion of the fuel phase containing emulsion was washed with distilled water and lyophilized. The lyophilized powder floated when placed again in distilled water.

Experiments were run to determine some of the chemical parameters which affect the tendecy of this contaminant to enter the fuel phase. This tendency was shown to be decidedly pH dependent. Figure 27 reveals that the emulsion enters the organic phase at regions of hydrogen ion concentration at which microbial growth is minimal, but yet these regions are not extreme in acidity and alkalinity. A mechanism of contamination may be visualized in which microbes growing around pH 7 synthesize the emulsion, and then with the cessation of growth or with changes in hydrogen ion concentration, it is brought into proximity with or concentrated at the fuel-water interface.

TABLE 5

OXYGEN UPTAKE OF FUEL AND WATER LAYER CELLS OF CULTURE 96 SUSPENDED IN BUFFER OR NBH

Substrate	Suspending Medium			
	NBH		Buffer	
	Fuel layer $\mu l/O_2$	Water layer $\mu l/O_2$	Fuel layer $\mu l/O_2$	Water layer $\mu l/O_2$
Fuel	31	35	14	20
Glucose	21	33	26	35

TABLE 6

GROWTH OF CULTURE 101 ON PURE HYDROCARBONS

Hydrocarbon	Time (Days)					pH	
	0	1	2	3	6	Initial	Final
Hexadecane	4×10^4	1×10^6	3.5×10^6	6.1×10^6	*	7.0	6.5
P-Cymene	4.5×10^4	3×10^5	2.4×10^5	4.5×10^5	6×10^5	6.9	6.6
M-Xylene	4×10^4	-	-	-	-	7.1	7.05
Decane	3.3×10^4	1×10^6	1×10^8	9×10^8	4×10^8	6.9	6.9
Cumene	5×10^4	3.3×10^2	$.3 \times 10^2$	2.2×10^5	3.1×10^4	7.1	7.0
Nonane	3.4×10^4	2.7×10^5	4.7×10^5	1.6×10^5	9×10^5	7.2	7.0
O-Xylene	3.6×10^4	-	-	-	-	7.1	7.0
1,2,4 Trimethyl benzene	3.8×10^4	3×10^3	3.1×10^3	3.3×10^5	9×10^4	7.1	6.9
Octane	3.5×10^4	3.5×10^5	1×10^7	2.3×10^7	2.2×10^7	7.2	6.9

*Flask broken

Inoculum per flask consisted of 5 ml of pure culture 101 which had been grown 48 hours at 30°C and washed 3 times in distilled H_2O. Each test flask contained 100 ml of NBH plus 10 ml of hydrocarbon substrate. Immediately following inoculation, inoculated flasks and sterile controls were placed in a rotary shaker at 30°C.

TABLE 6 (Cont.)

GROWTH OF CULTURE 101 ON PURE HYDROCARBONS

Hydrocarbon	Time (Days)					pH	
	0	1	2	3	6	Initial	Final
2,2,4 Trimethyl-pentane	1×10^4	1×10^5	7×10^4	1×10^5	[illegible] in excess of 10^7	7.2	6.6
Undecane	1.6×10^4	2×10^5	4×10^4	6×10^5	1.7×10^6	7.2	6.8
2-Hexene	no growth	-	-	-	-	7.2	7.1
2-Pentene	2×10^4	2.1×10^5	9×10^5	1.6×10^6	2.1×10^6	7.2	6.9
1-Heptene	2.5×10^3	-	-	-	-	7.2	6.9
Mesitylene	2.5×10^4	1×10^3	7×10^2	3.7×10^4	3.4×10^4	7.2	6.8
Pentane	4×10^4	7.9×10^4	3.5×10^5	1.2×10^6	6.1×10^6	7.2	6.9
1-Hexadecene	1.8×10^4	1.3×10^5	1.6×10^7	1.8×10^7	1.6×10^8	7.2	6.7
1-Octene	1.9×10^4	4×10^2	-	-	-	7.2	6.9
P-Xylene	2.9×10^4	-	-	-	-	7.2	6.9

TABLE 6 (Cont.)

GROWTH OF CULTURE 101 ON PURE HYDROCARBON

Hydrocarbon	Time (Days)						pH	
	0	1	2	3	6	34	Initial	Final
1-Tetradecene	1.5×10^6	5×10^8	1.3×10^9	1.3×10^9	1.8×10^9	8×10^7	7.2	4.0
Methylcyclo-pentane	1.7×10^6	3.3×10^4	7.7×10^5	8×10^5	1.4×10^6	2×10^4	7.2	6.8
1-Decene	1.7×10^6	1.4×10^9	2.69×10^9	8×10^9	5.5×10^9	2×10^9	7.2	4.7
2-3 Dimethyl butane	2.2×10^6	7.2×10^6	7×10^6	6.4×10^6	7.1×10^6	1.4×10^6	7.2	6.9
Tetradecane	1.3×10^6	5.2×10^5	8×10^7	3×10^8	2.08×10^8	1.2×10^8	7.2	5.81
Tridecane	2.1×10^6	4.2×10^6	5.7×10^6	9.7×10^7	1.1×10^7	1.6×10^7	7.2	5.75
Dodecane	1.9×10^6	5.1×10^7	2.5×10^8	4.5×10^8	4×10^8	6.1×10^7	7.2	3.2
1-Dodecene	1.9×10^6	8.3×10^7	2.5×10^8	4×10^8	4.6×10^8	1.6×10^8	7.2	3.7
Methylcyclo-hexane	1.5×10^6	1.7×10^6	8×10^6	8.4×10^7	1.7×10^8	5×10^7	7.2	6.7
2,2,5-Trimethyl hexane	1.9×10^6	5.0×10^6	5.4×10^6	8.3×10^6	7×10^6	1.3×10^6	7.2	6.9

Figure 27. The Dispersion of Microorganisms as a Function of pH in a Hydrocarbon Water System

The production of such emulsions is very important in microbial fuel contamination because emulsions represent a microbial product which is actually taken up by the fuel layer. Therefore, emulsions are a product whose chemical action is not confined to the limited area of the water bottom where microbial generation of fuel contaminants apparently takes place.

In a further effort to characterize the reactants which cause cell flotation and emulsion formation, determinations were made of the organic ester content of chloroform methanol extracts of cells which sediment, and of those cells which are not sedimentable in water. Using the hydroxalamine ferric perchlorate reaction on a unit cell weight basis, the cells which sought the fuel layer contained 50% more ester than those which were homogeneously dispersed in aqueous media.

In the future gas chromatography studies will be used to follow the microbial formation of these lipids, and the hydrocarbons which act as substrates will be sought.

2. The Contamination of Jet Fuel by Metabolic Products

In general in this laboratory the fuel isolates that have been grown in Bushnell-Haas medium with a jet fuel overlay have not produced colored compounds that are soluble in jet fuel. Fuel extractable compounds(s) were produced, however, in cultures grown at a high-nitrate concentration with nitrate as the only source of nitrogen.

The compound produced under these conditions was yellow; the absorption spectrum of this compound in fuel and in water at two extremes of pH are shown in Figure 28. The compound entered the fuel layer at pH 7 and was easily extracted into water and concentrated by adjusting the pH to 11.5. In aqueous solution the compound showed a definite inflection point as a function of pH; hence, it has a weak acidic functional group which exercise an inductive effect on its chromophore. Advantage was taken of the pH dependence of the extinction coefficient and a spectrophotometric titration of the compound was made. For this titration a mixed buffer containing 0.1M Tris buffer and NaOH adjusted to the indicated pH level with HCl was used. Figure 29 shows the results of this determination and sets the pH of the functional group controlling color change at pK 10.8. It seems probable that this group also controls the water solubility of the compound. Hence, these determinations show that this microbially produced fuel contaminant will begin to enter the fuel layer when the aqueous hydrogen ion concentration diminishes to values which permit microbial growth.

The yellow color of the compound and the fact that it is produced in media high in nitrate concentration suggest that it is a nitrated hydrocarbon. Preliminary experiments indicated that nitrated organic molecules may catalyze the corrosion of aluminum alloys. In media where this fuel extractable compound appeared in the fuel phase, aluminum corrosion was consistently observed in the water phase. This would indicate that the microbes might cause corrosion by producing a compound which is active in water at extremely low concentrations.

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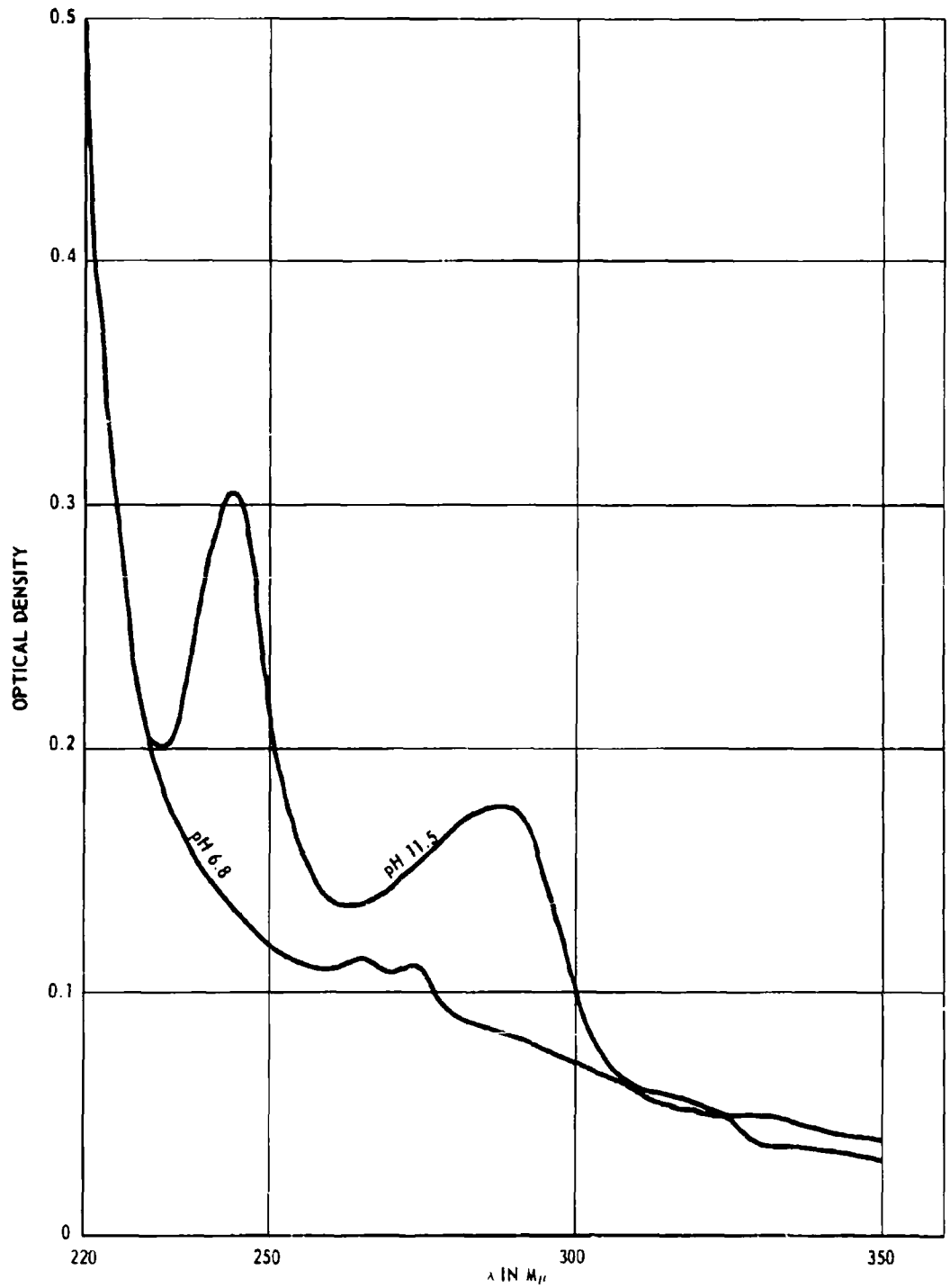


Figure 28. The Absorption Spectra of a Fuel Extractable Compound at pH 6.8 (Fuel Phase) and pH 11.5 (Water Phase)

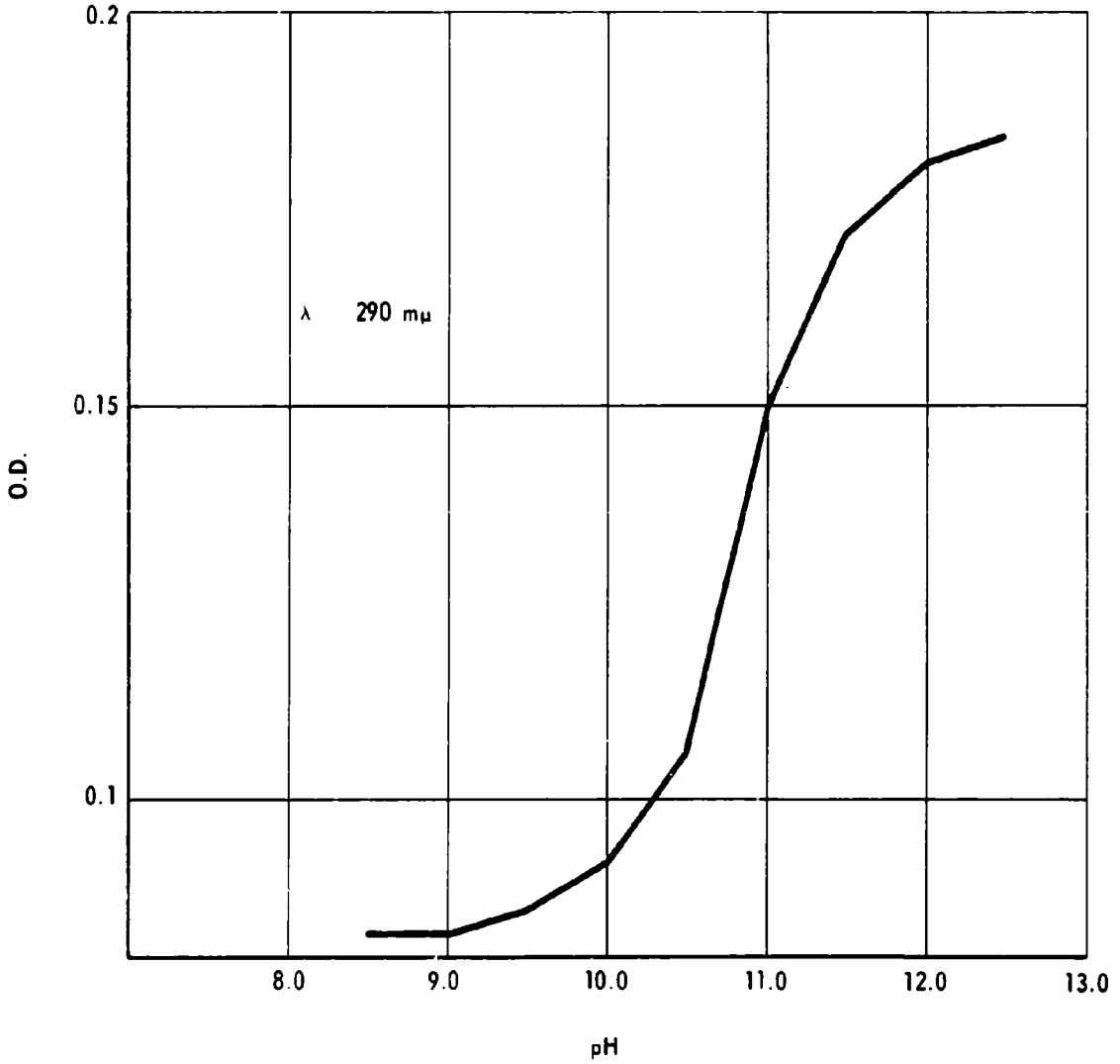


Figure 29. The Spectrophotometric Titration of a Fuel-Extractable Compound

3. The Microbial Oxidation of Fuel in Different Environments

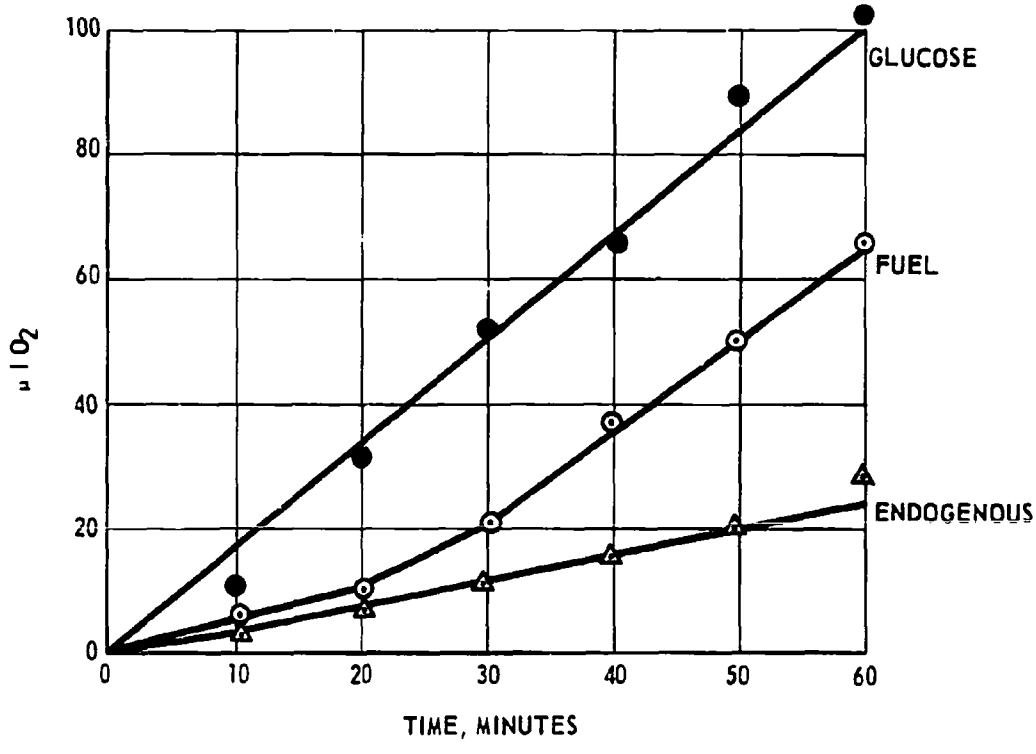
Some fundamental questions, to be answered in the study of fuel oxidation, concern the mode of adaptation of fuel isolates to hydrocarbon oxidation. Knowledge was sought of possible metal cofactor requirements for hydrocarbon oxidation. The value of this research in attempting to control microbial growth in fuel-water systems appears to lie in the possibility of finding inhibitors that would prevent the initial enzymatic oxidation of hydrocarbons. Such inhibitors would be especially effective in controlling microbial growth in fuel-water systems containing few alternate carbon sources. It is not unlikely that the enzymes which oxidize different hydrocarbons have chemically similar active sites and that they would be equally affected by a competitive or noncompetitive inhibitor. Thus, the use of such compounds would prevent bacterial growth on a wide variety of hydrocarbons.

In the study accomplished during this phase of the work on fuel oxidation, the Warburg apparatus was used to follow oxygen uptake as a function of time. The experiments were designed to show the effect of growth in different media on the ability of two fuel isolates to oxidize different carbon sources. Other experiments were accomplished to determine the effect of mineral media and phosphate buffer on the oxidation of jet fuel. Thus, two variables important to the metabolic capacity of the fuel isolates were considered: (a) previous growth media and (b) immediate ionic environment prevailing during fuel oxidation. Two substrates were chosen to investigate (a) the extremely metabolically inert hydrocarbons of fuel and (b) the extremely metabolically active sugar, glucose. The organisms used were the fuel isolates, Culture 96 and Culture 101. As discussed above, the former culture grew in clumps and made a fuel contaminant in the form of an emulsion at the fuel-water interface; the latter culture grows evenly dispersed through the aqueous phase of the fuel-water system.

These cultures were grown both on TGY and on Bushnell-Haas medium without nitrate (NBH). Following growth, they were centrifuged, washed, and re-suspended in phosphate buffer or in a solution of minerals of NBH. The two cultures differed considerably in their oxidative responses just as they differed in their growth characteristics.

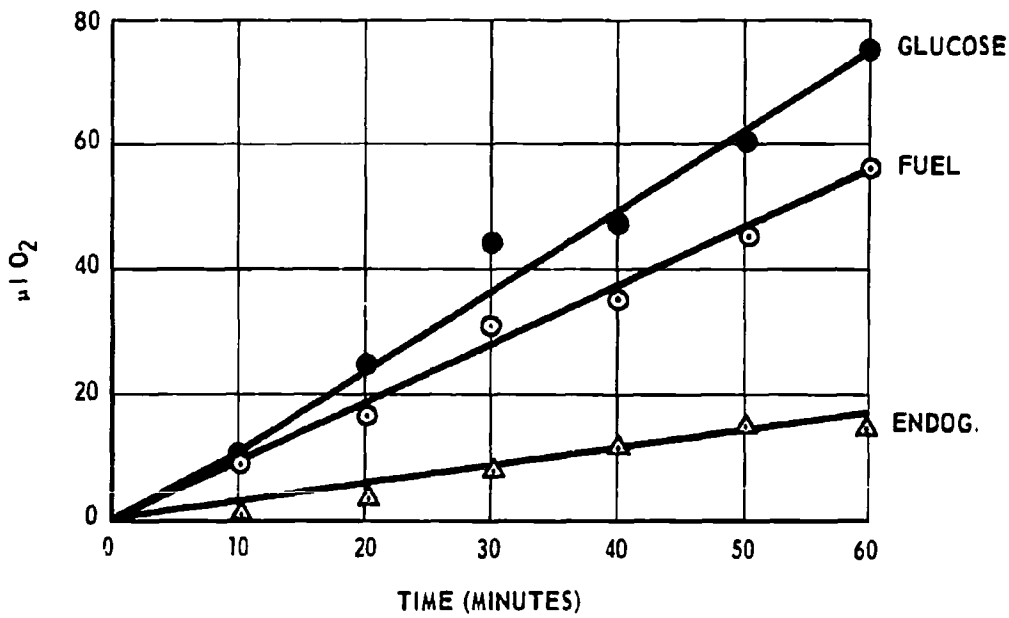
Cells of Culture 96 grown on TGY were adapted to carbohydrate oxidation and utilized glucose at a constant rate as shown in Figure 30; these cells were unadapted to the oxidation of fuel, but became adapted within 30 minutes following exposure to fuel. Culture 101 grown on TGY was adapted, both to fuel and to glucose oxidation, and oxidized both substrates rapidly during a 1-hour period (Figure 31). Culture 101 oxidized fuel at approximately 75% the rate at which it oxidized glucose.

Both organisms were grown on NBH with a jet fuel overlay, and they were harvested and suspended either in phosphate buffer or in NBH without fuel. In the absence of the minerals of the NBH medium, cells of Culture 96 were unable to sustain a constant rate of fuel oxidation (Figure 32). After 60



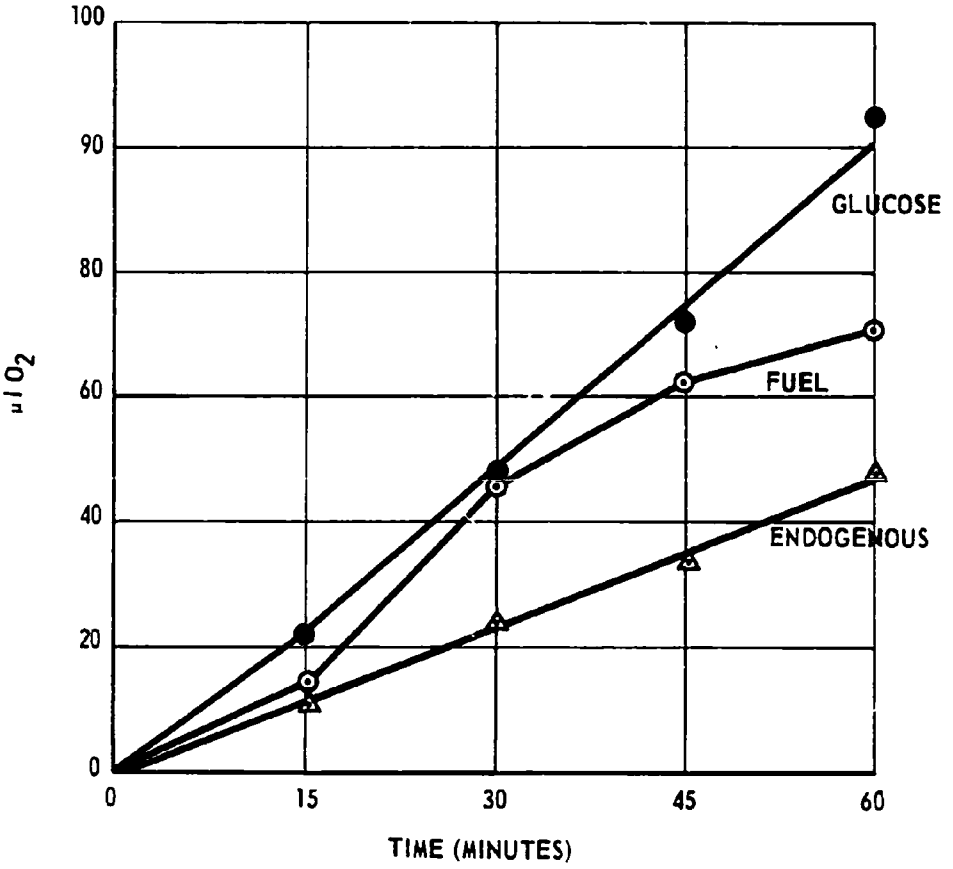
FLASK CONTENTS: 1.0 ML CELLS (3.4×10^9/ML), 0.5 ML FUEL OR 1% GLUCOSE OR BH SALT SOLUTION, 1.5 ML BH SALT SOLUTION; CENTER WELL 0.2 ML 25% KOH.

Figure 30. The Oxidation of Glucose and JP-4 Fuel by TGY Grown Culture 96 in BH Medium



FLASK CONTENTS: 1.0 ML CELLS (5.3×10^9/ML), 1.5 ML BH-SOLUTION, 0.5 ML FUEL, or 1% GLUCOSE, OR BH-SOLUTION. CENTER WELL 0.2 ML 20% KOH.

Figure 31. The Oxidation of Glucose and JP-4 Fuel by TGY Grown Culture 101 in BH Medium



FLASH CONTENTS: 1.0 ML CELLS (8 X 10^9/ML), 1.5 ML 10^{-2}/M K_2HPO_4 BUFFER pH 7.0, 0.5 ML FUEL, OR 1% GLUCOSE, OR BUFFER. CENTER WELL 0.15 ML 20% KOH.

Figure 32. The Oxidation of Glucose and JP-4 Fuel in Buffer by NBH-Grown Culture 96.

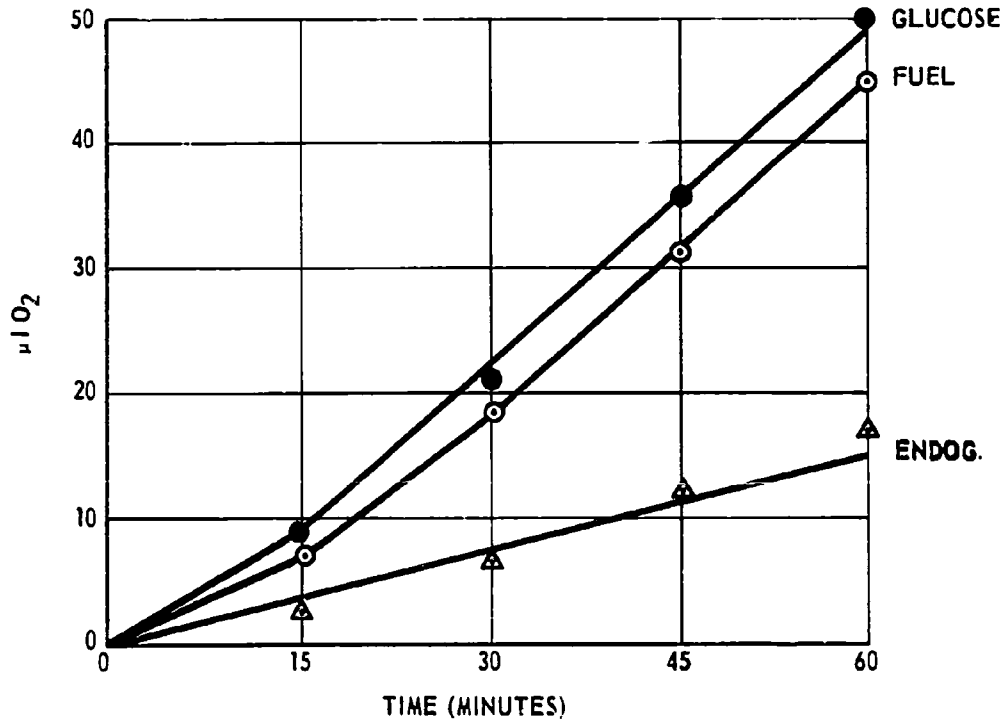
minutes in phosphate buffer alone, their ability to oxidize fuel was essentially lost. But when cells of Culture 96 were washed in NBH medium and exposed to fuel in the Warburg, they oxidized fuel rapidly and sustained their initial rate of oxidation (Figure 33). The response of Culture 96, grown on fuel, again contrasted with that of Culture 101 grown on fuel. Fuel-grown cells of the latter culture were adapted to the oxidation of jet fuel, Figure 34, and oxidized it rapidly even when suspended in only phosphate buffer as data not presented here indicated. It appears that the mineral content of the suspending medium is not of great consequence to the oxidative ability of Culture 101, but it is of considerable importance to the oxidation of fuel by Culture 96. This finding shows that the control of the mineral content of a medium may lead to the control of emulsion formation.

Studies were performed to determine what mineral constituents contributed most to the oxidation of hydrocarbons by whole cells. The fuel isolate, Culture 96, was used in these investigations. As shown above, if fuel-grown organisms of this strain are washed in phosphate buffer, their ability to oxidize jet fuel was diminished to about 75%. However, with the addition of the various minerals of the growth medium to cells suspended in buffer, as shown in Figure 35, Ca^{+2} produced the greatest stimulation of hydrocarbon oxidation; following this, Mg^{+2} and Fe^{+3} stimulated hydrocarbon oxidation. During a 135-minute period of respiration measurement, the rate of oxygen uptake with phosphate alone continuously diminished while the respiratory rate with each of the metals tested, alone and in combination, increased. The similarity of the stimulation produced by both di- and trivalent ions suggest that the effect observed is nonspecific rather than an effect on the active center of the hydrocarbon oxidizing enzyme. An explanation of this stimulation may be sought in terms of alterations to permeability of the bacteria to the hydrocarbon substrate.

Research on metal requirements for the oxidation of hydrocarbons will be continued using cell extracts from the fuel-oxidizing organism. These studies will be designed to determine the importance of permeability to the inhibition and stimulation of hydrocarbon oxidation observed with whole cells. Cell extract studies will facilitate the analysis of the effect of Al^{+3} and other ions of aluminum alloys on the activities of hydrocarbon decomposing enzymes, and on corrosion initiation by oxidation products formed by these enzymes. Using organisms that are obtained in abundance from the rich medium, attempts will be made to find the contribution of various enzymes to the problem of jet fuel contamination.

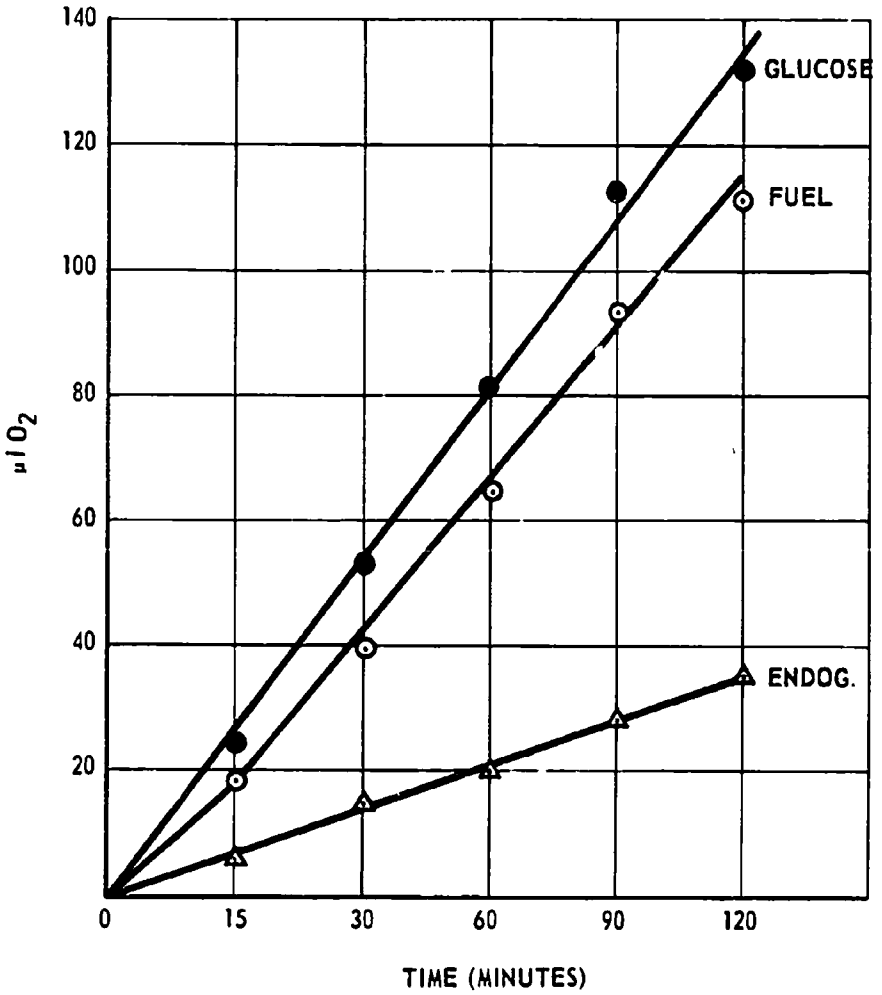
4. The Inhibition of the Microbial Oxidation of Jet Fuel by Hydrocarbons and by Known Respiratory Inhibitors

The character of metabolic mechanisms responsible for particular chemical transformations carried out by microorganisms can frequently be elucidated by the use of compounds which inhibit normal physiological activities. For example, if the activity of proteolytic enzymes of fuel isolates caused the corrosion of aluminum reported in this study, the use of inhibitors which



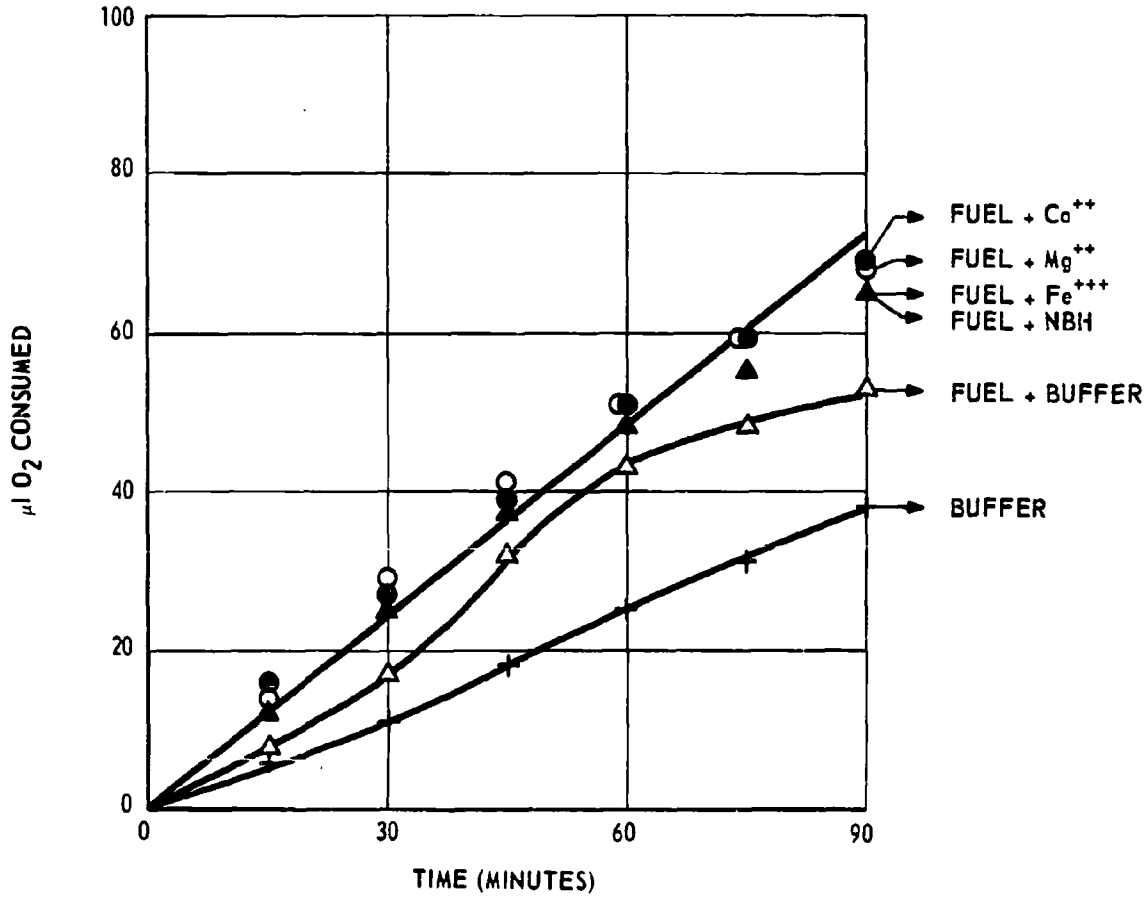
FLASK CONTENTS: 1.0 ML CELLS (8 X 10^9/ML), 1.5 ML 10^{-2} M K_2HPO_4 BUFFER, pH 7.0, 0.5 ML FUEL, OR 1% GLUCOSE, OR BUFFER. CENTER WELL 0.15 ML 20% KOH.

Figure 33. The Oxidation of Glucose and JP-4 Fuel in BH-Medium by NBH-Grown Culture 96.



FLASH CONTENTS: 1.0 ML CELLS (3.7×10^9/ML)
1.5 ML BH-MEDIUM, 0.5 ML FUEL, OR 1% GLUCOSE,
OR BH-MEDIUM. CENTER WELL 0.15 ML 20% KOH

Figure 34. The Oxidation of Glucose and JP-4 Fuel in BH-Medium by NBH-Grown Culture 101



FLASK CONTENTS: 1.0 ML CELLS (2.8 X 10^{10}/ML); 1.0 ML 10^{-2}M PHOSPHATE BUFFER, pH 7.1, OR 1.0 ML 10^{-3}M Ca, Mg, Fe AS THE CHLORIDES OR 1.0 ML NBH MEDIUM. CENTER WELL 0.2 ML 25% KOH. REACTION RUN AT 37°C.

Figure 35. The Effect of Mg^{++}, Ca^{++}, and Fe^{+++} on the Oxidation of Fuel by Culture 96

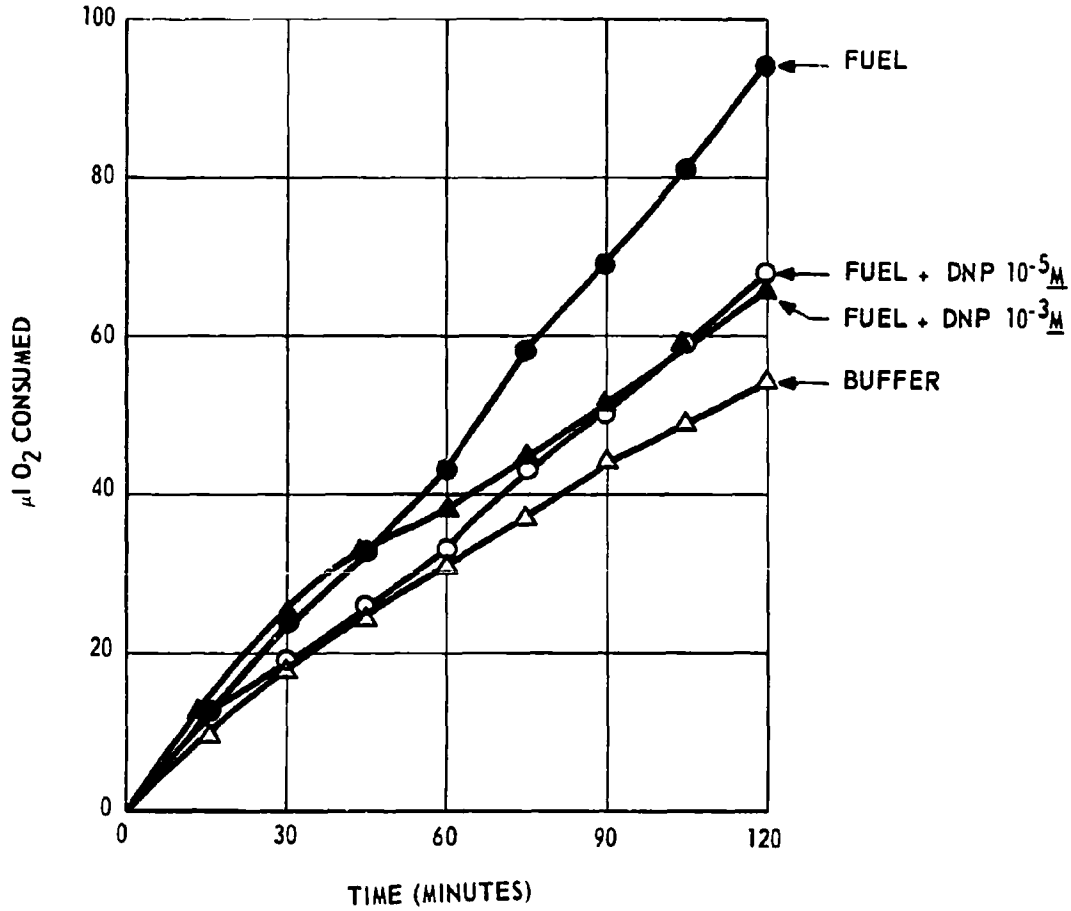
prevented the action of this class of catalyst would prevent corrosion. The observation that these known inhibitors prevented corrosion would constitute evidence of the type of mechanism responsible for corrosion. The application of this approach (form of reasoning) to an understanding of the physiological mechanisms operative in jet fuel oxidizing organisms has been attempted.

It was shown above that some fuel isolates were capable of oxidizing hydrocarbons without an adaptation period while others require an extended period of exposure to hydrocarbons before the ability to oxidze them was acquired. It was also shown that the presence of metal ions was essential to the sustained oxidation of hydrocarbons by some strains, and that others could bind these metals in such a manner that their presence in the external medium was not required for oxidation. These data suggest that microbial activity on jet fuel could be greatly reduced by the presence in water bottoms of compounds which are known to prevent microbial adaptation. The metal requirement shown in these studies, reported for in vitro conditions in the literature,[3,4] suggest that the enzymes responsible for fuel oxidation and bacterial respiration contain metals. Sodium azide inhibits the activity of metal containing enzymes and, like 2,4-dinitrophenol, it prevents adaptation.

Tests were made of the effect of respiration inhibitors on the activity of fuel isolates. The anabolic inhibitor 2,4-dinitrophenol which stops adaptation, is effective at concentrations as low as 10^{-5} M against some organisms. The data in Figure 36 show that concentrations of this compound as high as 10^{-3} M do not appreciably affect the ability of jet fuel organisms to oxidize hydrocarbons. The resistance of these organisms to dinitrophenol, as judged by respiration studies, implies that they are either impermeable to the compound or that they possess a mechanism for oxidative phosphorylation which is unique among bacterial systems. Dinitrophenol is insoluble in fuel at pH's near neutrality, and the addition of this compound to fuel systems would result in its concentration in the water phase of a water-fuel system. There, it would hopefully prevent microbial activity. Because of the resistance of fuel isolates, however, the use of dinitrophenol does not appear to be recommended as a fuel additive having biocidal activity.

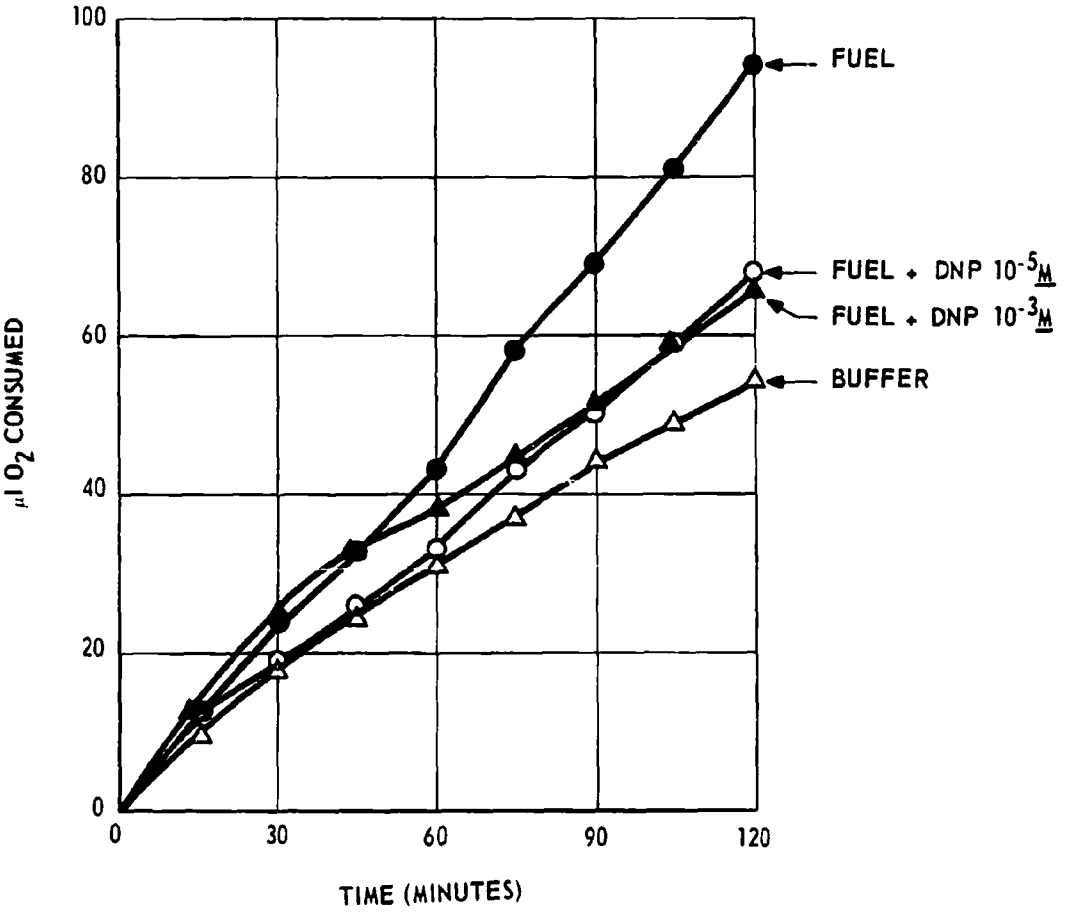
It appeared probable that metal-containing enzymes played an important role in (1) the rapid initial oxidation of hydrocarbons to fuel contaminants like organic peroxides or carboxylic acids and (2) the catalytic mechanisms which permit the conversion of primary products, such as carboxylic acids to carbon dioxide and water and lower acids. Thus sodium azide might be expected to react with the metal in either enzyme. This seemed especially probable since the metal involved appears to be iron, but here again the metabolic activity of the fuel isolates proved to be surprisingly atypical and resistant. Even 10^{-3} M azide inhibited respiration only 30% (Figure 37). This cellular response indicates that the terminal oxidase may be a flavin-containing enzyme rather than a cytochrome.

Figures 38a & 38b show that the initial step in fuel oxidation was probably mediated by a metal-containing enzyme because fuel oxidation was inhibited by the chelating agent, EDTA. The lack of dependence of carbohydrate oxidation on extracellular minerals was shown again for this organism.



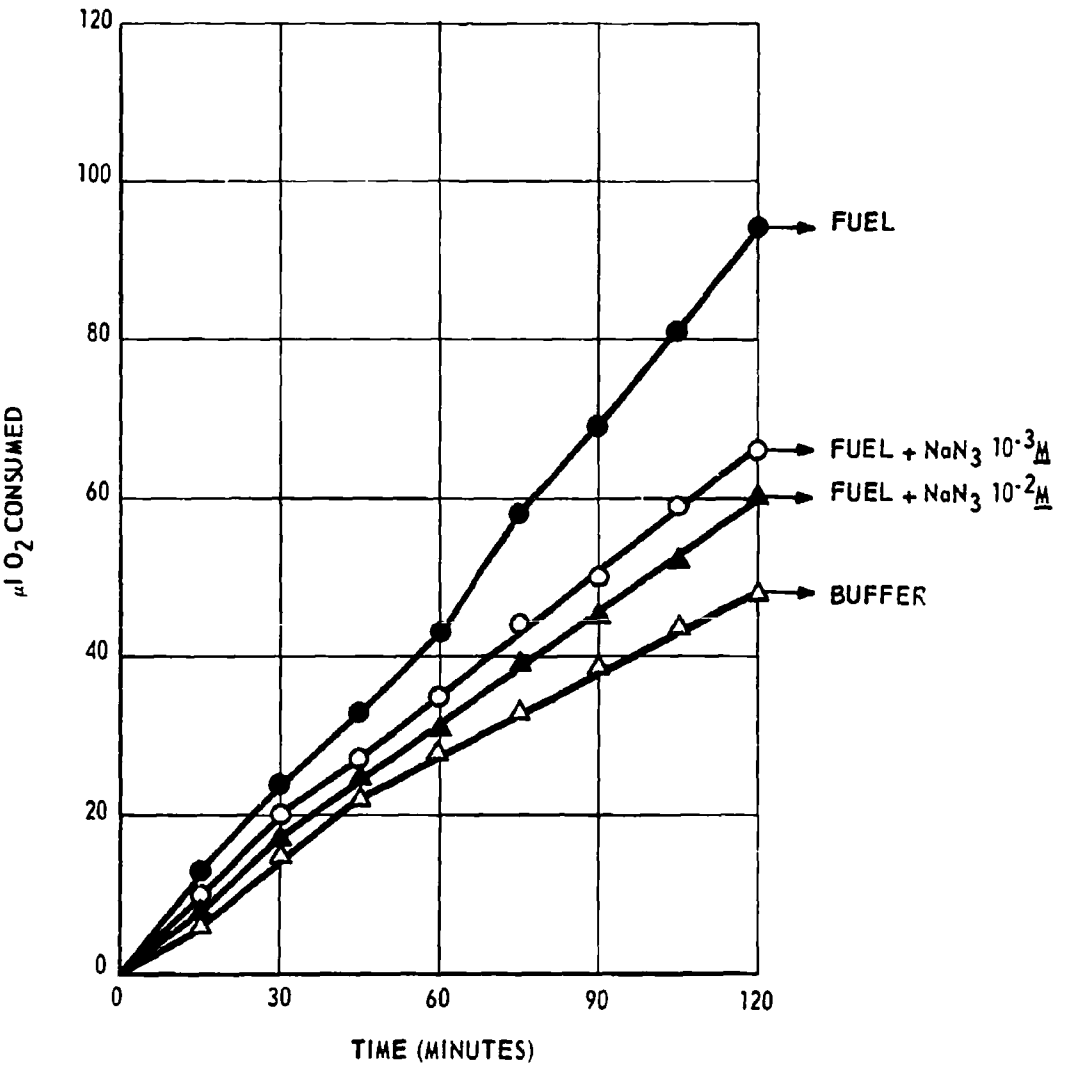
FLASK CONTENTS: 1.0 ML CELLS (2.1 X 10^{10}/ML) 0.5 ML FUEL; 1.0 ML 10^{-2}M PHOSPHATE BUFFER, pH 7.1; 0.5 ML DNP 6 X 10^{-3}M; CENTER WELL 0.2 ML 25% KOH. REACTION RUN AT 37°C.

Figure 36. The Effect of 2,4-Dinitrophenol on Jet Fuel Oxidation by Jet Fuel Isolates



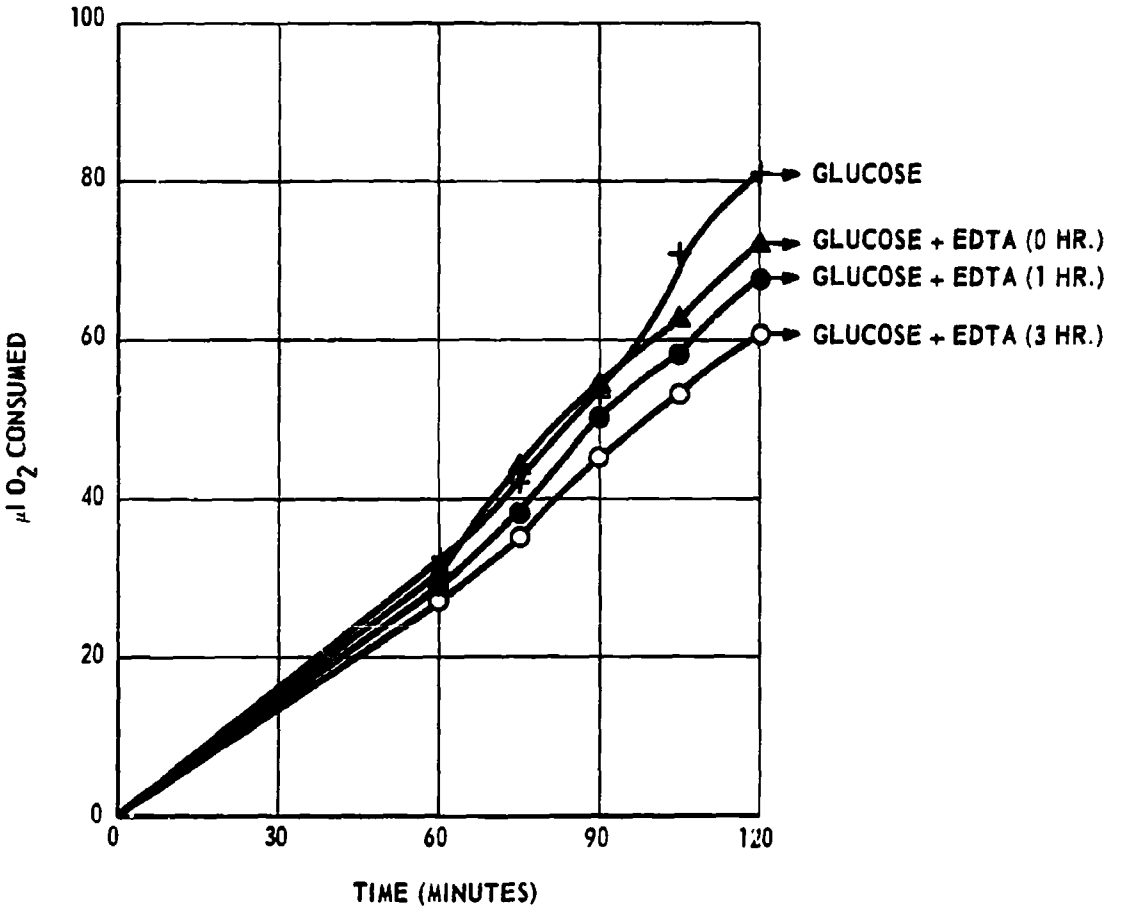
FLASK CONTENTS: 1.0 ML CELLS (2.1 X 10^{10}/ML) 0.5 ML FUEL; 1.0 ML 10^{-2}M PHOSPHATE BUFFER, pH 7.1; 0.5 ML DNP 6 X 10^{-3}M; CENTER WELL 0.2 ML 25% KOH. REACTION RUN AT 37°C.

Figure 36. The Effect of 2,4-Dinitrophenol on Jet Fuel Oxidation by Jet Fuel Isolates



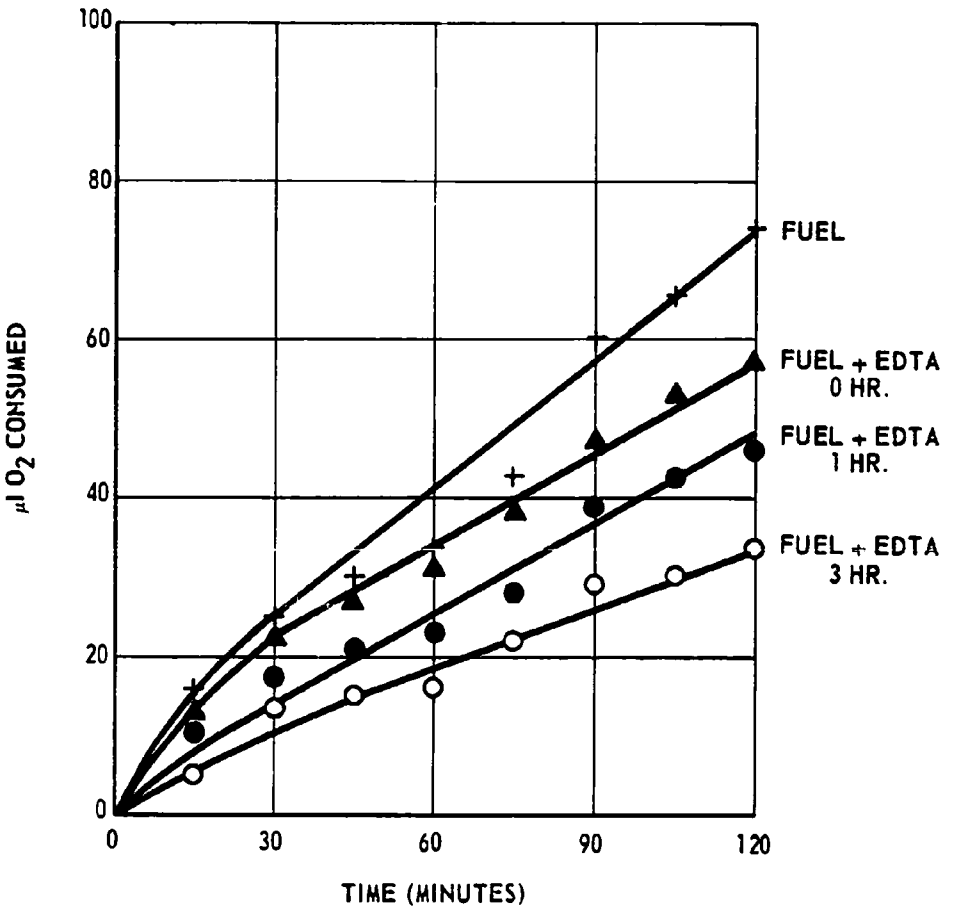
FLASK CONTENTS: 1.0 ML CELLS (2.1 X 10^{10} ML); 0.5 ML FUEL; 1.0 ML 10^{-2}M PHOSPHATE BUFFER, pH 7.1; 0.5 ML NaN_3 6 X 10^{-3}M OR PHOSPHATE BUFFER; CENTER WELL 0.2 ML 25% KOH. REACTION RUN AT 37°C.

Figure 37. The Effect of NaN_3 on the Oxidation of Jet Fuel by Jet Fuel Isolates



FLASK CONTENTS: 1.0 ML CELLS (5.9 X 10^9/ML); 0.5 ML FUEL OR GLUCOSE: 1.0 ML 10^{-2}M PHOSPHATE BUFFER, pH 7.1; 0.5 ML EDTA TO MAKE FINAL CONC. OF 2.5 X 10^{-2}M OR 0.5 ML BUFFER; CENTER WELL 0.2 ML 25% KOH. REACTION RUN AT 37°C. EDTA INCUBATED WITH CELLS IN FLASKS 0, 1, AND 3 HOUR INTERVALS BEFORE DETERMINATION WAS RUN.

Figure 38a. The Effect of EDTA on the Oxidation of Jet Fuel by a Fuel Isolate



FLASK CONTENTS: 1.0 ML CELLS (5.9 X 10^9/ML); 0.5 ML FUEL OR GLUCOSE; 1.0 ML 10^{-2}M PHOSPHATE BUFFER, pH 7.1; 0.5 ML EDTA TO MAKE FINAL CONC. OF 2.5 X 10^{-2}M OR 0.5 ML BUFFER; CENTER WELL 0.2 ML 25% KOH. REACTION RUN AT 37°C. EDTA INCUBATED WITH CELLS IN FLASKS 0, 1, AND 3 HOUR INTERVALS BEFORE DETERMINATION WAS RUN.

Figure 38b. The Effect of EDTA on the Oxidation of Glucose by a Fuel Isolate

The two types of inhibitors studied above were not studied as potential additives to fuel, but only for their usefulness in defining the mechanism of hydrocarbon oxidation.

The oxidation of compounds compatible with fuel which exert a definite physiological effect on microbial contaminants were studied. The data in Table 6 showed that organisms did not grow on hexene, heptene, and p-xylene, and it was shown in other experiments that hexane and neptane neither supported the growth of fuel isolates nor were they bacteriacidal to them. In this respiration study the ability of organisms to oxidize these compounds was studied as well as their effect on the oxidation of fuel and of glucose.

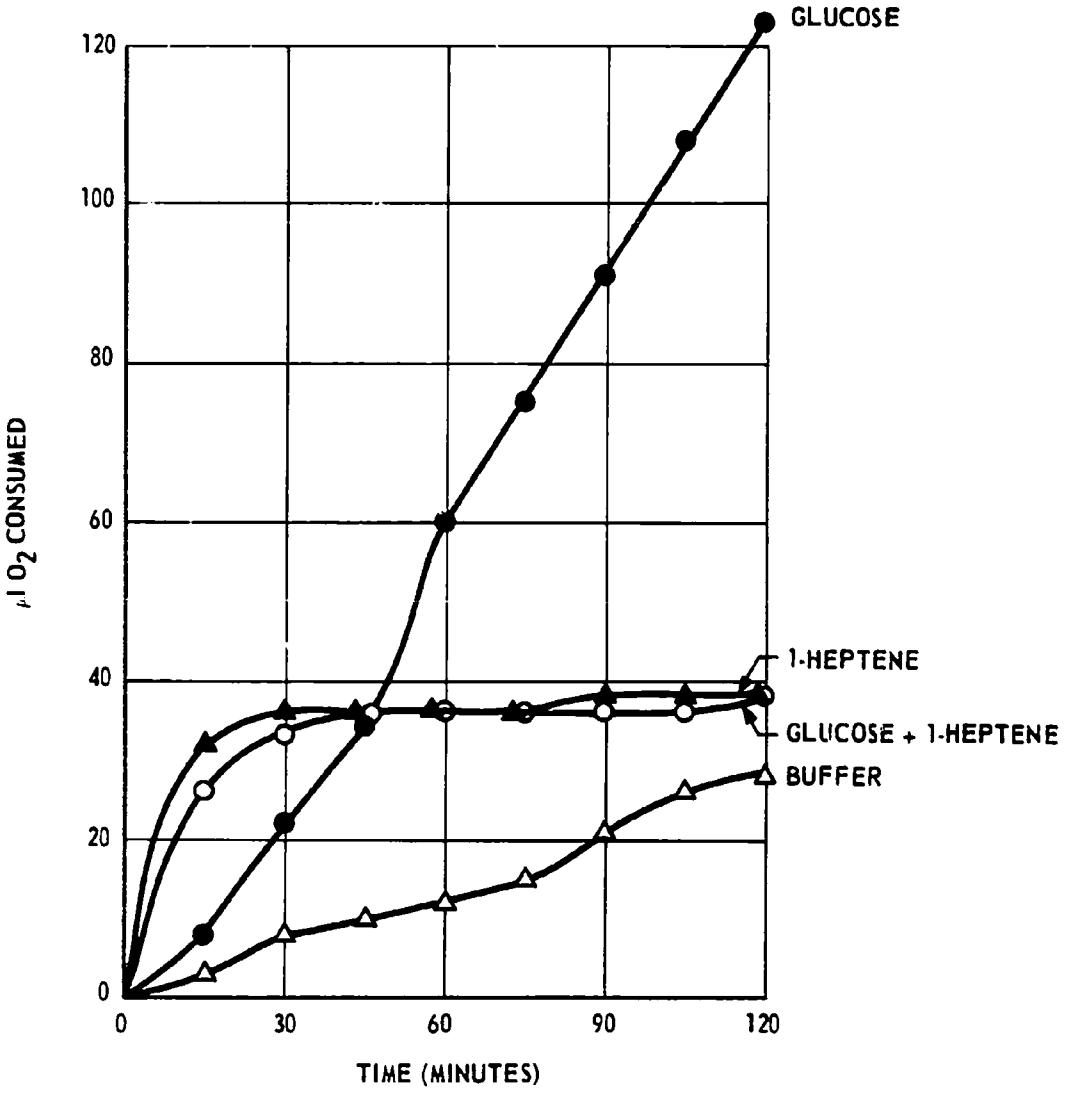
Figures 39a and 39b show the inhibition of jet fuel and glucose oxidation by 1-heptene. It is of interest that neither 1-heptene nor heptane are oxidized appreciably. However, heptane does not prevent the oxidation of fuel while 1-heptene stops fuel oxidation and the oxidation of glucose. The same pattern of effects was found for hexane and 2-hexene, Figure 40a and 40b. Both octane and nonane are oxidized (Figure 41), but other data not included here showed that neither the one nor the other inhibits fuel or glucose oxidation.

These respiration studies show that carbon length becomes critical with respect to alkane oxidation in the transition from seven to six carbon atoms, while the presence of one double bond in six and seven carbon compounds causes them to become inhibitors of fuel oxidation.

Unsaturated compounds, such as 1-heptene and 2-hexene, may alter microbial fuel contaminants in a variety of ways. They are similar to long chain alkanes and may compete with fuel for the active sites of fuel oxidative enzymes. The presence of a double bond makes the molecule more polar than the homologous saturated structure, and it may displace the saturated hydrocarbon from the enzyme surfaces. These short-chain unsaturated compounds surprisingly prevent the oxidation of carbohydrates-like glucose, as well as fuel hydrocarbons. This observation implies that a larger part of the cell structure was affected than just the hydrocarbon oxidizing system. This belief was supported further by the observation that small olefin molecules kill hydrocarbon oxidizing microbes as well as prevent their respiration. This generalized effect was evident also with p-xylene . Figure 42 shows that fuel oxidation in the presence of p-xylene was prevented, and other data have shown that p-xylene was not, itself, oxidized, and killed organisms which were isolated from fuel systems.

This study of metabolic mechanisms emphasizes the complexity of factors controlling the appearance and activity of fuel contaminants. The results obtained show the presence of areas of unexpected resistance and sensitivity in the physiological makeup of organisms which inhabit fuel-water system. The information obtained gives an understanding of the biochemical mechanisms operative in microbial contamination and at the same time indicates means by which these contaminants may be controlled.

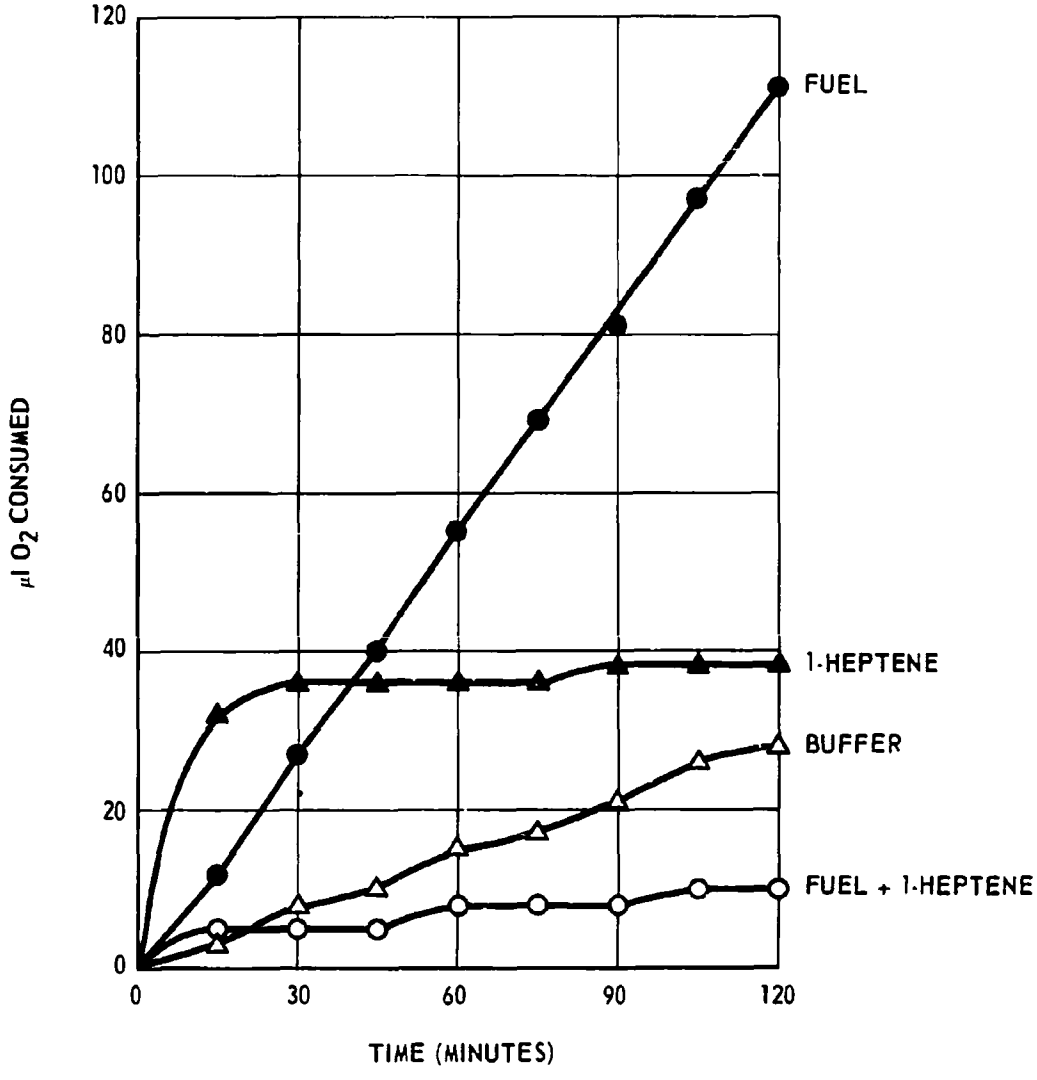
R9059A



FLASK CONTENTS: 1.0 ML CELLS (8.9 X 10^9/ML); 0.5 ML GLUCOSR OR FUEL; 1.0 ML 10^{-2}M PHOSPHATE BUFFER, pH 7.1; 0.5 ML 1-HEPTENE OR BUFFER; CENTER WELL 0.2 ML 25% KOH. REACTION RUN AT 37°C.

Figure 39a. The Effect of 1-Heptene on Glucose Oxidation by Culture 101

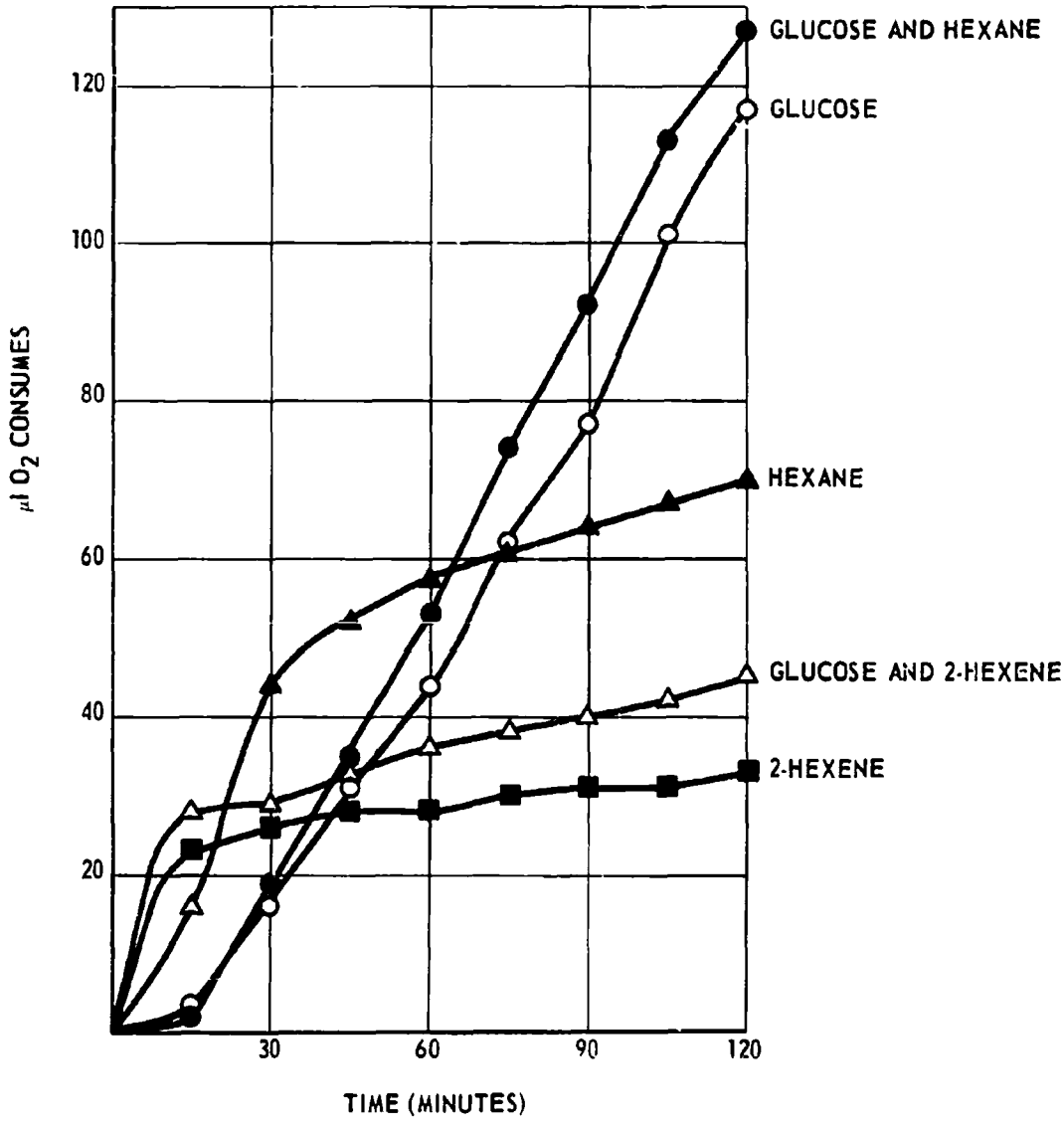
R9059B



FLASK CONTENTS: 1.0 ML CELLS (8.9 X 10^9/ML); 0.5 ML GLUCOSE OR FUEL; 1.0 ML. 10^{-2}M PHOSPHATE BUFFER, pH 7.1; 0.5 ML 1-HEPTENE OR BUFFER; CENTER WELL 0.2 ML 25% KOH. REACTION RUN AT 37°C.

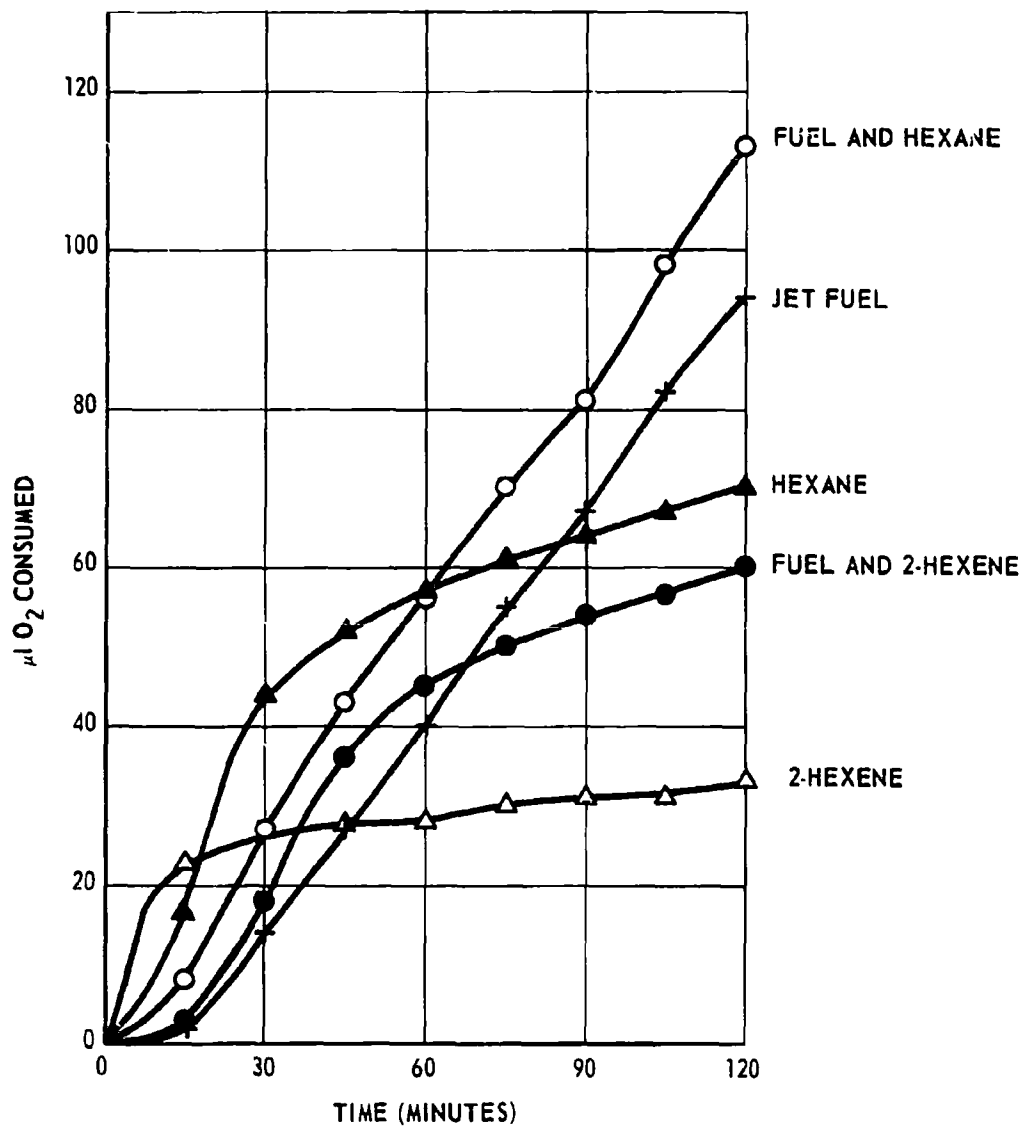
Figure 39b. The Effect of 1-Heptene on Jet Fuel Oxidation by Culture 101

R9060A



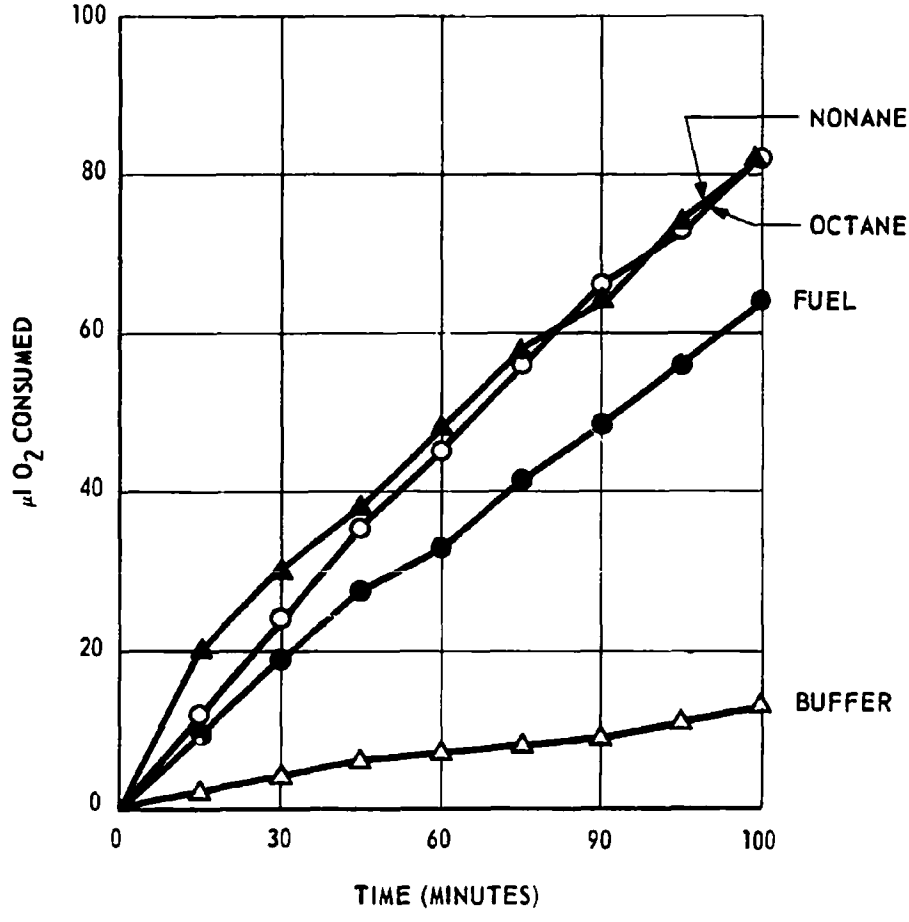
FLASK CONTENTS: 1.0 ML CELLS (1.4 X 10^{10}/ML); 1.0 ML 10^{-2}M PHOSPHATE BUFFER, pH 7.1; 0.5 ML FUEL OR GLUCOSE; 0.5 ML HEXANE OR 2-HEXENE OR BUFFER; CENTER WELL 0.2 ML 25% KOH. REACTION RUN AT 37°C.

Figure 40a. The Effect of Hexane and 2-Hexene on the Oxidation of Glucose by Culture 101



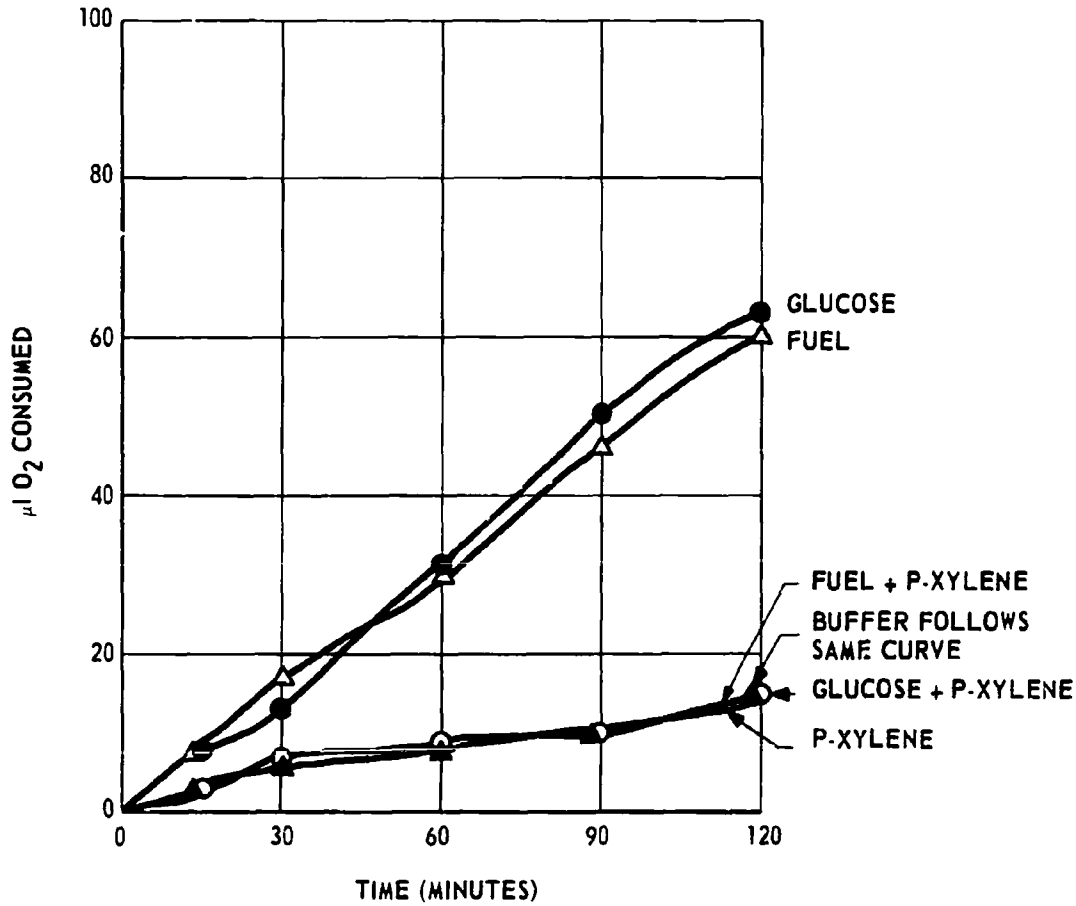
FLASK CONTENTS: 1.0 ML CELLS (1.4 X 10^{10}/ML); 1.0 ML 10^{-2}M PHOSPHATE BUFFER, pH 7.1; 0.5 ML FUEL OR GLUCOSE; 0.5 ML HEXANE OR 2-HEXENE OR BUFFER; CENTER WELL 0.2 ML 25% KOH. REACTION RUN AT 37°C.

Figure 40b. The Effect of Hexane and 2-Hexene on the Oxidation of Jet Fuel by Culture 101



FLASK CONTENTS: 1.0 ML CELLS (10^{10}/ML); 1.0 ML 10^{-2}M PHOSPHATE BUFFER, pH 7.1; 1.0 ML FUEL, OCTANE, OR NONANE; CENTER WELL 0.2 ML 25% KOH. REACTION RUN AT 37°C

Figure 41. The Oxidation of Octane, Nonane, and Jet Fuel by Jet Fuel Isolates



FLASK CONTENTS: 1.0 ML CELLS (9.2 X 10^9/ML); 0.5 ML GLUCOSE OR FUEL; 1.0 ML 10^{-2}M PHOSPHATE, BUFFER, pH 7.1; 0.5 ML P-XYLENE OR BUFFER; CENTER WELL 0.2 ML 25% KOH. REACTION RUN AT 37°C.

Figure 42. The Effect of P-Xylene on the Oxidation of JP-4 Fuel and Glucose by Culture 101

5. The Killing of Fuel Isolates by Hydrocarbons

The appearance of microbial contaminants in jet fuel intuitively suggests that the organisms present are capable of oxidizing hydrocarbons as a source of energy. Also, many components of fuels are known to be protein denaturants, and it may be assumed that organisms which survive in fuel possess a special property of resistance to these agents which distinguishes them from other bacteria. Studies were made to attempt to distinguish fuel-oxidizing organisms from those which cannot oxidize fuel on the basis of the resistance of the former and the sensitivity of the latter to the possible lethal effects of exposure to fuel when suspended in a saline or a growth medium.

It was hypothesized that fuel-oxidizing cells entered fuel systems either as cells adapted to growth on rich media or as cells adapted to growth on fuel-containing media, while the fuel-water bottom may possess a mineral content ranging from predominately sodium chloride to that of a complete growth medium in terms of trace metals and nitrogen source. In this phase of the study fuel-oxidizing organisms (Culture 96) were grown both on a modified Bushnell-Haas medium with a fuel overlay and on TGY. The cells were harvested by centrifugation and were washed and suspended in either NaCl or H_2O. The washed cells were then inoculated into NaCl, H_2O, or BH with a fuel overlay. Cells in each medium grew during exposure for 4 days to fuel.

The results obtained (Table 7) show the extreme hardiness of the organisms which oxidize fuel, and, though a limited sample was analyzed, the viability of this particular fuel-oxidizing organism did not appear to be strongly dependent on the kind or concentration of salts in the water phase of its environment. It was observed that a very large fraction of these cells grown on TGY survive when placed in the presence of fuel. This suggests that growth on fuel is a characteristic of a large part of the bacterial population and does not derive from a few mutant organisms among the TGY-grown cells. The ability of the cells grown on a rich medium to adapt to the fuel medium suggests the importance of organic contaminants in fuel-water bottoms to the establishment of large microbial populations in fuel systems which can oxidize fuel upon exhausting other sources of carbon.

Cells of Escherichia coli were grown on TGY (tryptone-glucose-yeast extract) medium and treated in every way similar to the fuel isolates. In 4 days the viability of the E. coli suspension diminished to 0.03% to 0.06% of its initial value; but the viability of Culture 96 remained essentially unchanged during this period (Table 7). The viability of E. coli and Culture 96 in saline and distilled water without fuel was essentially unchanged during this 4-day period. Essentially the same results were obtained with nitrogen-deficient medium where the cell count remained constant in absence of growth for 97 days. These results show that fuel components are toxic to the nonfuel-oxidizing organism, E. coli, but not to fuel organisms. A question is raised concerning the mechanism by which fuel isolates are protected from killing by fuel; is it due to permeability or is it due to enzymatic composition of the cells?

TABLE 7.

THE EFFECT OF JP-4 FUEL ON THE VIABILITY OF E. COLI AND A FUEL ISOLATE

Inoculum*	Media	Initial Count	Percent Survival		
			0 time	2 days	4 days
E. coli	Distilled H_2O Fuel	2.8×10^8	100%	0.075%	0.0679%
	NBH-fuel	3.6×10^8	100%	0.75%	0.01%
96	Distilled H_2O Fuel	2.14×10^7	100%	91.6%	78.5%
	NBH-fuel	1.98×10^7	100%	51%	17.18%
96	Distilled H_2O No fuel	1.3×10^6	100%	146%	615%
E. coli	Distilled H_2O No fuel	3.8×10^8	100%	105.5%	150%
E. coli	Saline fuel	3.67×10^8	100%	0.0329%	0.039%
	NBH-fuel	2.27×10^8	100%	0.0538%	0.031%
96	Saline-fuel	3.1×10^7	100%	10.98%	8.9%
	NBH-fuel	1.65×10^7	100%	16.96%	13.34%
E. coli	Saline - no fuel	2.5×10^8	100%	52%	76.9%
96	Saline - no fuel	1.1×10^6	100%	276%	255%
96 (Saline washed cells)	Saline-fuel	6.0×10^2	100%	966.6%	7,166.6%
	NBH-fuel	4.0×10^2	100%	20,000%	73,333%
96 (Distilled H_2O washed cells)	Distilled H_2O - fuel	4.5×10^2	100%	3111%	7333%
	NBH-fuel	2.2×10^2	100%	42,636%	190,909%

*All cells were grown in TGY.

Respiration studies had shown that 1-heptene, 2-hexene, and p-xylene are potentially toxic to fuel-oxidizing organisms; these compounds stop fuel oxidation. The first experiments to determine the mechanism by which fuel isolates are protected from killing by fuel involved the effect of the testing of the viability of fuel isolates in the presence of these unsaturated hydrocarbons.

Washed cells of Cultures 96 or 101 were inoculated in Bushnell-Haas medium with an overlap of a test compound alone or dissolved in various proportions in JP-4 fuel. The cultures were brought to 28°C, and samples were taken for a viability count at the end of different time periods. Table 8 summarizes the effect on the survival of three cultures after 6 hours of exposure to hydrocarbon overlays with various proportions of inhibitor and jet fuel. The curves in Figures 43, 44, and 45 show respectively the time course inactivation of Culture 101 by an overlay of 1-heptene, 2-hexene, and p-xylene. The complexity of the curve suggests that death results at different concentrations of toxin from different mechanisms; hence, water-soluble compounds possessing unsaturated hydrocarbon moieties may be capable of exercising a large measure of control over the entire oxidative activities of fuel microbes as well as their growth and reproduction.

Survival appeared to be a strong function of the proportion of fuel present. Only a small difference in response of the three cultures was noted to any hydrocarbon. 1-heptene appeared to be the most toxic compound at large ratios of 1-heptene to JP-4 fuel, but some overlap was apparent with 2-hexene present at smaller proportions. P-xylene was decidedly less toxic than the unsaturated compounds, but it nevertheless showed that certain hydrocarbons can penetrate the fuel isolates to kill them by mechanisms similar to that occurring with normal cells. Future studies will be concerned with determining whether normal fuel will inhibit the respiratory activity of cell free extracts of normal and fuel cultures. This study should demonstrate whether the cell enzymes are inactivated by fuel in which case the protective role of the cell membrane would be proven.

TABLE 8

THE PERCENT SURVIVAL OF CULTURE 96, CULTURE 101, AND A MIXED CULTURE AFTER EXPOSURE TO 1-HEPTENE, 2-HEXENE, AND P-XYLENE

Added Hydrocarbon	Jet Fuel	Organism	% Survival after 6 Hours 1-Heptene	2-Hexene	p-Xylene
0.05ml	10 ml	96	>100	80	>100
1.00	10	96	90	35	>100
5.00	10	96	75	12	90
10.00	10	96	3	<1	50
10.00	0	96	<1	<1	14
0.05	10	Mixed	100	---	>100
1.00	10	Mixed	98	---	>100
5.00	10	Mixed	84	---	80
10.00	10	Mixed	4	---	50
10.00	0	Mixed	1	---	22
0.05	10	101	100	>100	>100
1.00	10	101	90	>100	>100
5.00	10	101	52	30	75
10.00	10	101	1.5	< 1	40
10.00	0	101	< 1	< 1	7

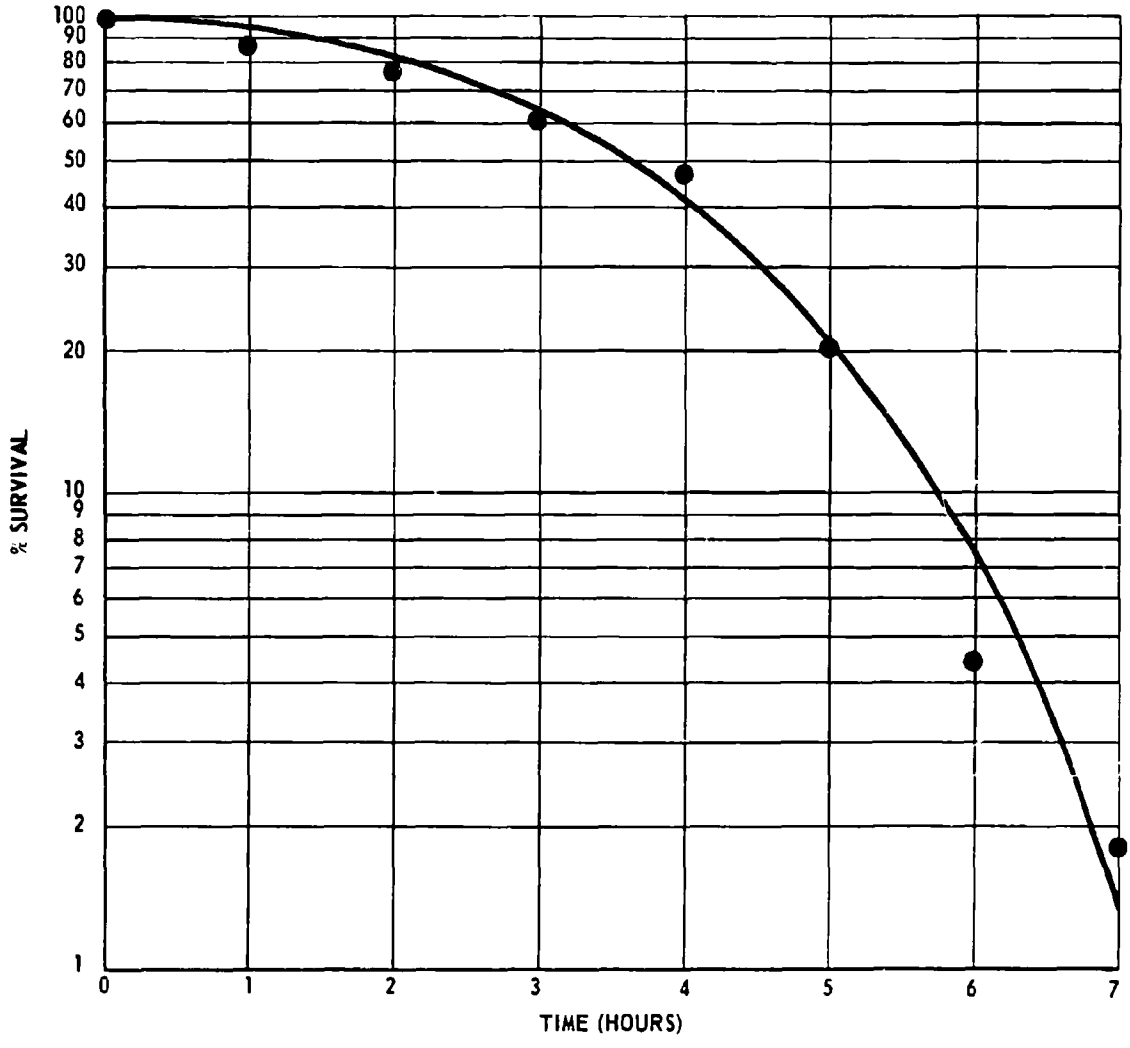


Figure 43. Survival of Culture 101 as a Function of Time in Media with an Overlay of 1-Heptene

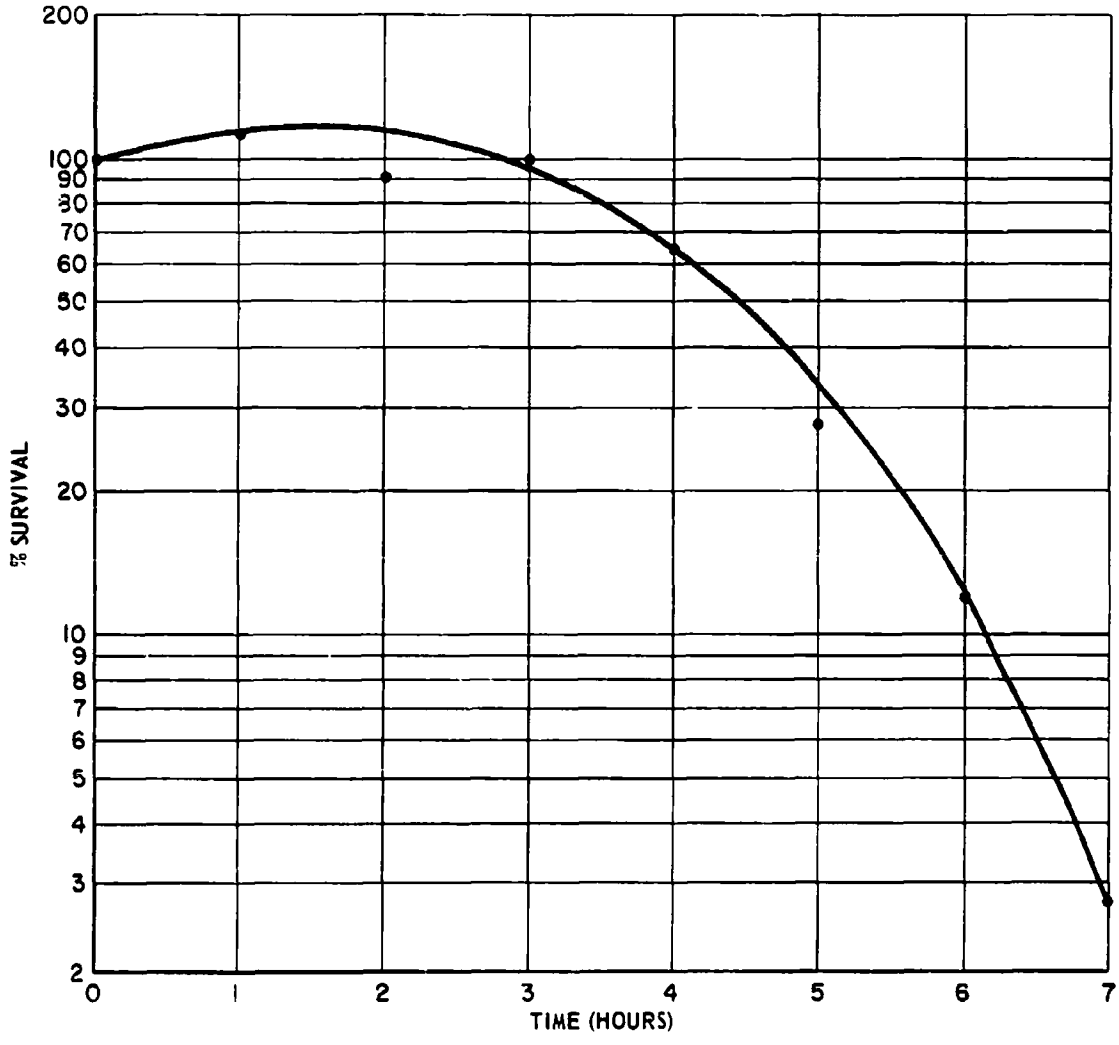


Figure 44. Survival of Culture 101 as a Function of Time in Media with an Overlay of 2-Hexene

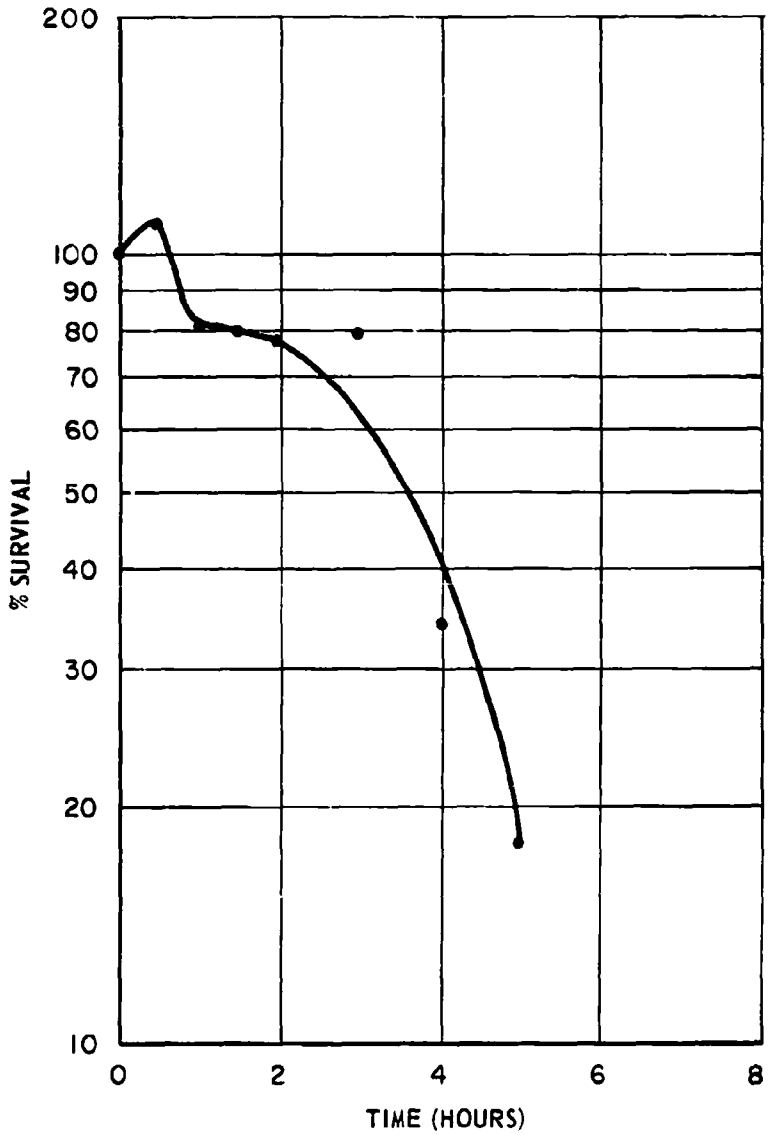


Figure 45. Survival of Culture 101 as a Function of Time in Media with an Overlay of P-Xylene

C. The Microbial Deterioration of Coatings and Sealants*

Experiments designed to demonstrate that microorganisms are capable of utilizing topcoatings and sealants as a source of either nitrogen or carbon are described. The data indicate that all of the coatings tested are utilized to some extent by microorganisms, but that the degree of utilization varies greatly. The coatings may be roughly classified into three groups: (a) not measurably utilized, (b) probably utilized, and (c) readily utilized.

Coatings on aluminum coupons incubated in the presence of microorganisms, mineral salts, and fuel have started to fail. There is a clear correlation between failure of one of the coatings and microbial growth.

Assays indicated that three out of eleven coatings contained water extractable growth inhibitors. Two appeared to be transitory, but chromate polymerized polysulfide was consistently inhibitory.

1. Microbial Deterioration of Coatings

A number of topcoatings and sealants are mentioned in this report. Generic names plus a code number have been used throughout the report. Table 9 presents a list of the generic terms and the code numbers used in this report. The products used are identified in a separate letter.

2. Nitrogen and Carbon-free Media Studies

This experimental design is based on the use of coatings as the sole source of either nitrogen or carbon in media inoculated with a mixed culture of bacteria. In time the bacteria should adapt to the use of coatings as a source of either nitrogen or carbon and provide cultures that are able to rapidly deteriorate coatings.

The microorganisms used were 15 unidentified cultures recently isolated from a coated water system. These specific cultrures were used since they had been growing in pure water in contact with a coating compound. It is also commonly believed that cultures that have not been carried on artificial media for long periods of time are more adaptable to new environments.

The medium used as a carbon-free substrate was a simple mineral salts solution using ammonium salts as a nitrogen source. The nitrogen-free medium substituted dextrose for the nitrogen. The composition of the media is presented in Table 10.

Difficulty has been experienced using this approach. The basal media supported growth without coatings, and the results were confused. However, improved data have been obtained using exhausted media as noted in the following procedure:

---

* Work performed by Sharpley Laboratories, Inc.

TABLE 9

TEST COATINGS

Generic Description	Identification Number
Buna N type coatings	
Sample 1	Buna-1
Sample 2	Buna-2
Sample 3	Buna-3
Sample 4	Buna-4
Polysulfide-type coatings and sealants Chromate catalyzed	
Sample 1	Polysulfide-1
Sample 2	Polysulfide-2
Manganese dioxide catalyzed	
Sample 1	Polysulfide-3
Polyurethane-type coatings	
Sample 1	Polyurethane-1
Sample 2	Polyurethane-2
Furan type	
Sample 1	Furan-1
Metal-containing type	
Sample 1	Metal-1

TABLE 10

NITROGEN AND CARBON-FREE MEDIA

Carbon-free medium

Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.4	g
Calcium chloride ($CaCl_2$)	0.02	g
Potassium phosphate, dihydrogen (KH_2PO_4)	2.0	g
Potassium phosphate, monohydrogen (K_2HPO_4)	2.0	g
Ammonium phosphate ($NH_4H_2PO_4$ -$(NH_4)_2HPO_4$- 1:1)	0.599	g
Distilled water, q.v.	1000	ml

Nitrogen-free* medium

Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.4	g
Calcium chloride ($CaCl_2$)	0.02	g
Potassium phosphate, dihydrogen (KH_2PO_4)	2.0	g
Potassium phosphate, monohydrogen (K_2HPO_4)	2.0	g
Dextrose	1.0	g
Distilled water, q.v.	1000	ml

* Label analyses indicate 7.8 ppm N as contaminants. Chemical analysis gave a mean of 4.03 mg N/liter.

Exhaust of Media

To remove the trace nutrients, batches of media in Fernbach flasks were inoculated with a mixed culture and agitated for 3 to 5 days at 28°C. The cells were removed by filtration through a 0.3 μ pore size membrane filter.

Growth Tests

The test coating was polymerized and cured on siliconed glass; it was then removed and chopped as finely as possible for use.

Cells were harvested from bottle slants and washed three times. Four percent of the resulting slurry was used as an inoculum in either carbon-free or nitrogen-free media. The cells were not carried on normal media after this point. This was substrate 1. Incubation was at 28°C, agitated.

After 4 to 7 days, the cells were counted and removed. The coatings were washed with 250 ml of water, three separate times. Preincubated, exhausted substrate was added and 4.0% of the cells from substrate 1 were used as an inoculum. This was substrate 2.

After 4 to 7 days, the procedure was repeated, providing substrate 3.

This procedure did not prevent growth in the controls, but the ratio of growth between the media containing coatings and the controls gradually moved to favor the former. These data are presented in Tables 11 and 12. Inspection of Table 11 allows the following conclusions to be drawn concerning nitrogen utilization in the third substrate:

a. Growth equal to or greater than the control was obtained using every extracted coating in the third substrate.

b. Less than a twofold increase in growth 0 to 2X of the control growth over the control was obtained with:

Buna - 3
Polymethane 2 - not extracted
Polyurethane - 1
Buna - 2 extracted
Polysulfide 1
Buna - 4
Buna - 2

TABLE 11

GROWTH OF BACTERIA ON NITROGEN-FREE MEDIA

	Nitrogen-free media Bacteria/ml/x10^7					
	1st Substrate		2nd Substrate		3rd Substrate	
	Ext	Not Ext	Ext	Not Ext	Ext	Not Ext
Polyurethane-2	38	31	3	2.8	4.7	2.0
Buna-3	32	53	4.7	4.0	1.9	2.6
Polyurethane-1	37	39	2.1	1.7	2.4	2.6
Metal-1	100	130	45	100	18	55
Control	30		1.8		2.0	
Polysulfide-3	13	29	6.5	7.2	5.4	5.2
	11	11	4.5	2.0	3.7	3.1
833-5	7.2	15	3.1	2.0	2.0	2.7
Control	20		2.2		2.4	
Polysulfide 890	8.1	9.2	2.75	2.8	1.8	1.1
Control	5.8		1.4		1.0	
Furan-1	26.3	20	5.3	6.1	1.0	0.58
Polysulfide-2	26.7	30.6	4.5	4.4	0.52	0.66
Polyurethane-2	28.3	30.2	2.8	4.7	0.47	0.63
Buna-1	10.9	19.1	9.4	11	0.69	1.1
Control	25.6		0.91		0.22	

Notes:

1. All counts are direct microscopic counts using a Petroff-Hauser Chamber. Cell viability confirmed by plate count. Incubation 4 to 7 days, agitated at 28°C.
2. 2nd and 3rd substrates are adapted cells. Evaluations on basis of 3rd substrate. Each control applies only to the preceding group of experiments. See text for further definitions.
3. "Ext" in the sub-headings indicates that the coating had been extracted in boiling distilled water after polymerization and curing. "Not Ext" indicates the coating was used as is.

TABLE 12

GROWTH OF BACTERIA ON CARBON-FREE MEDIA

	Carbon-free media Bacteria/ml/x10^7					
	1st Substrate		2nd Substrate		3rd Substrate	
	Ext	Not Ext	Ext	Not Ext	Ext	Not Ext
Polyurethane-2	31	31	1.2	1.4	0.27	0.34
Buna-3	32	52	3.1	23	2.3	9.3
Polyurethane-1	50	48	12	15	7.2	8.1
Metal-1	52	67	44	71	12	91
Control	22		0.88		0.25	
Polysulfide-3	9.1	14	0.94	6.1	1.4	1.05
Buna-4	11	33	3.1	77	2.0	23
Buna-2	16	8.9	3.5	22	0.7	15
Control	7.0		0.7		0.27	
Polysulfide 890	6.1	6.6	1.1	1.3	1.2	1.6
Control	3.4		0.97		0.88	
Furan-1	18.3	91.9	4.1	13	0.74	6.1
Polysulfide-2	22.5	22.2	5.2	8.0	0.96	3.3
Polyurethane-2	35.5	27.5	2.5	.64	0.44	0.39
Buna-1	20.3	12.8	2.2	7.2	1.7	5.3
Control	14.8		0.2		0.13	

Notes:

1. All counts are direct microscopic counts using the Petroff-Hauser Chamber. Cell viability confirmed by plate count. Incubation 4 to 7 days, agitated at 28°C.
2. 2nd and 3rd substrates are adapted cells. Evaluations on basis of 3rd substrate. Each control applies only to the preceding group of experiments. See text for further definitions.
3. "Ext" in the sub-headings indicates that the coating has been extracted in boiling distilled water after polymerization and curing. "Not Ext" indicates the coating was used as is.

c. Up to a fourfold increase in growth from 2X to 4X of the control growth was obtained with the following:

Polyurethane - 2 extracted
Polysulfide - 3
Furan - 1
Polysulfide - 2
Buna - 1

d. A great increase in growth was recorded for:

Metal - 1

These data are supported by the nitrogen analyses in Tables 13 and 14. All of the water extracts of coatings contained nitrogen and this is reflected in the growth pattern in nitrogen-free media. Nitrogen fixation was suspected, but the data in Table 14 indicate that it is not likely in these cultures.

With reference to the growth tests using carbon-free media, the conclusions below may be drawn from the data in Table 12. The same protocol was followed as outlined for the nitrogen growth tests.

a. Growth on the third substrate was better with all coatings than the control without coatings.

b. Less than a twofold increase in growth over the control was obtained with:

Polyurethane - 2
Polysulfide - 1

c. Up to tenfold increases in growth over the control were obtained with:

Polysulfide - 3
Buna - 4
Buna - 2
Furan - 1 extracted
Polysulfide - 2

d. Greater than tenfold increases in growth over the control were obtained with:

Buna - 3, not extracted
Polyurethane - 1
Metal - 1
Buna - 4, not extracted
Buna - 2, not extracted
Furan - 1, not extracted
Polysulfide - 2, not extracted
Buna - 1

TABLE 13

NITROGEN DETERMINATIONS ON EXTRACTS OF COATINGS

Water Extracts	Mg N/liter			Mean
Buna-4	8.0	.8.16	8.10	8.1
Metal-1	6.05	6.0	(10.8)	6.0
Polysulfide-3	37.6	35.6	36.0	36.4(2)
Buna-2	12.35	12.92	14.1	13.12
Polyurethane-1	8.20	8.40	8.88	8.46
Buna -1	11.80	9.48	9.80	10.35
Polyurethane-2	15.48	-----	14.40	14.94
Furan-1	3.68	4.02	-----	3.85

Notes:

1. Polymerized coatings extracted with boiling distilled water. Nitrogen determinations made on extracts by Hengar digestion and distillation followed by nesslerization. Accuracy about ±10%.

2. Order of magnitude only. Rechecked.

TABLE 14

NITROGEN DETERMINATIONS ON MEDIA

	Mg N/liter			Mean
"Nitrogen-free" media - Controls	4.0	4.09	4.0	4.03
"Nitrogen-free" media inoculated with Culture No.				
2		4.31		
3		4.76		
4		3.98		
5		1.32		
6		4.28		
7		4.16		
9		4.98		
10		5.12		
11		4.78		
12		4.42		
13		3.24		
14		4.06		
15		4.06		
Mixed - 2nd transfer		6.40		
Mixed - 3rd transfer		3.36		

Notes:

Determined by Hengar distillation and nesslerization.

Accuracy about ±10% at low levels when samples are replicated. Considerably less on single samples.

The interrelationships of the tests for nitrogen utilization are very interesting. Chemical analysis of water extracts from coatings all show significant amounts of nitrogen. The absolute quantities present would vary widely, it is suspected, if different extraction techniques were used. Interference is suspected on the tests with Polysulfide 1675 since the amount of nitrogen appears unreasonalby high.

Preliminary nitrogen determinations have been made on Buna-N coatings and found to be very high. This is not unexpected since the nitrogen analysis will presumably reflect the -CN group of acrylonitrile, i.e.,

$$-\left[-\left(CH_2-CH=CH-CH_2\right)_x-CH-CH_2-\right]_n-$$
$$\qquad\qquad\quad |$$
$$\qquad\qquad\quad CN$$

The source of nitrogen in polysulfide is puzzling if it is assumed that the polymer is $-\left[-CH_2-CH_2-\underset{\underset{S}{\|}}{S}-\underset{\underset{S}{\|}}{S}-\right]_n-$. But, specific information on chemical composition of coatings is difficult to obtain and there are apparently a number of ways of forming this polymer. In addition, all of these coatings are commercial compounds and presumably are prepared from commercial grade chemicals. Large amounts of trace materials may be introduced from this source.

With reference to the polyurethane materials, it is assumed that the Wurtz reaction using an isocyanate and an alcohol is the basis for the polymer. For example, a polyurethane polymer may be based on hexamethylene diisocyanate and butyl glycol. In this event, nitrogen will be available from either the N=C of the diisocyanate or the -NH- of urethane. Thus, the presence of nitrogen in extracts from these coatings is not surprising.

There is no apparent relationship between the amount of nitrogen found chemically in water extracts and the disc assay tests for toxicity. Other factors apparently override the nitrogen factor in a complete medium such as that used for the disc assay tests.

With reference to the nitrogen-free growth tests, there is usually little difference in growth between an extracted and nonextracted coating. The notable exception is Metal - 1 where the nonextracted coating gives a much better growth. The obvious assumption to make is that a growth-stimulating substance is removed by water extraction. This is partially confirmed by the disc assay tests, although stimulation on these tests is not nearly as clear-cut as inhibition.

As outlined previously, from two- to fourfold increases in growth was obtained with five coatings in the series. The lower numbers in this group have doubtful significance, but it seems probable the data on extracted Furan - 1 and Buna - 1 show significant utilization of the coating as a nitrogen source.

With reference to the various Buna-type coatings, it is significant that there are apparently differences in the coatings. These are not only reflected by the utilization studies, but the disc assay test indicates that a toxicant has apparently been added to Buna - 2, either intentionally or accidentally.

In general, the coatings tested may all be utilized to some extent as a nitrogen source by the mixed population of microorganisms used in these tests. The coatings fall in three groups. One group shows a twofold or less increase in growth that may or may not be significant. The second group shows a two-to-fourfold increase with probable increasing significance. The last group shows a great increase in growth and is highly significant.

The data concerning the utilization of coatings as the sole source of carbon contains a number of surprises. There has been much more emphasis placed on extractable nitrogen than carbon from coatings serving as a microbial growth stimulant. However, these data indicate that very significant growth occurs at the expense of carbon from the coatings.

There are no cases where growth in media containing chopped pieces of coatings is not greater than the controls. However, in view of the magnitude of growth obtained with most of the coatings, it appears highly significant that Polyurethane 1560 and Polysulfide 890 show little or no increase in growth.

A number of coatings showed greater than a tenfold increase in growth and it appears certain that these coatings are being used as a carbon source by the microorganisms. Extraction of the coatings is very important in these tests. Toxic materials may be removed by the water extraction or it is possible that the procedure makes the carbon more available to the microorganisms.

It is not known whether coatings will be utilized for carbon in the presence of hydrocarbon fuels. This will be difficult to determine in view of the usual excellent utilization of hydrocarbons as a carbon source; but it seems important in view of the data at hand.

3. Growth Affecting Substances in Coatings

Water soluble materials present in coatings may either stimulate or inhibit the growth of bacteria. The detection of such materials is of importance when evaluating growth tests and also as background information covering toxic additives.

The procedure used was to polymerize and cure the coating; the coating was then minced and 5 g extracted for 30 minutes in 100 ml of boiling distilled water. Discs were cut from the cast films and assayed both before and after extraction. Filter paper assay discs were saturated with the water extract for testing.

Melted and cooled TGY agar was inoculated with the mixed bacterial culture and poured into petri dishes. After solidification, extracted and nonextracted discs of coatings, and filter paper discs saturated with the water extracts, were placed on the surface of the agar. All tests were run in triplicate. Inhibition was judged to be present on the basis of a clear zone surrounding the test disc. Stimulation was recorded if a zone of increased growth either surrounded the disc or if there was a slight zone of inhibition surrounded by a zone of stimulation.

The data are reported in Table 15. Inspection of these data show the following:

a. Four Buna-N type compounds were tested and three exhibited stimulation of bacterial growth by the water extract. The fourth material contains inhibitory substances as defined in these tests.

b. Chromate polymerized polysulfide sealants (Polysulfide-2) was inhibitory in these tests.

c. The manganese dioxide polymerized polysulfide sealant stimulated growth.

d. Polyurethane-type topcoating material shows some stimulation of growth in these tests.

e. Water extracts from Furan-type materials are slightly inhibitory.

f. The metal-containing material (Metal-1) gave slight stimulation when the water extract was tested. The unextracted coating also stimulated growth and the extracted coating had no effect.

4. Preliminary Resistance Measurements

A large number of aluminum and steel coupons coated with various coatings are in the process of being tested as described in the next section. There appears to be a chance that these coupons may be used for resistance measurements and, thus, obtain double information from the tests. The purpose is to provide some type of measurement of coating deterioration other than visual estimates.

Estimates of the resistance was made using a simple Triplett ohmmeter. One lead was attached to a bare aluminum strip and the second was clipped to the coated coupon with a bulldog clip that penetrated the coating.

Preliminary data are presented in Table 16. It should be recognized that these measurements are rough estimates made with a simple test instrument. Differences have been obtained within some of the series and better instrumentation may produce significant differences.

TABLE 15

RESULTS OF DISC ASSAY TESTS ON COATINGS

Compound	Water Extract	Extracted Coating	Unextracted Coating
Furan -1	Inhibition	No effect	No effect
Polysulfide -1	No effect	Stimulation	Stimulation
Polysulfide -2	Inhibition	Inhibition	Inhibition
Buna -1	Stimulation	No effect	No effect
Buna -2	Inhibition	No effect	No effect
Buna -4	Stimulation	No effect	No effect
Polysulfide -3	Stimulation	Slight stimulation	Stimulation
Polyurethane -1	Stimulation	Slight stimulation	Slight stimulation
Metal -1	Slight stimulation	Slight stimulation	Stimulation
Buna -3	Stimulation	No effect	Stimulation
Polyurethan -2	Stimulation	No effect	Slight stimulation

TABLE 16

PRELIMINARY RESISTANCE MEASUREMENTS

Coating	Substrate	Inoculated	Resistance x 10^3
Buna -1	Steel	yes	0.7 0.9 1.2 0.7 0.8 1.2
		no	1.1 1.1
	Aluminum	yes	1.4
		no	1.1 1.2
Polysulfide -1	Steel	yes	1.9 2.0 1.8
		no	2.0 2.2
	Aluminum	yes	0.85 0.85 0.85
		no	0.85 0.85 0.85
Buna -2	Aluminum	yes	1.4 1.0 1.4
		no	1.6 1.4

Notes:

1. Approximate resistances obtained by "triplex" meter in electrolyte solution.

2. Buna -1 on steel have incubated 76 days, remainder about 44 days. Static incubation at 28° C.

5. Visual Inspection of Coated Panels

Detailed observations are recorded in Table 17, presenting the appearance of the aluminum and steel coupons with test coatings. Coatings were applied to coupons, polymerized, and cured. The coated coupons are incubated vertically in inoculated TS-11 medium, using JP-4 as a carbon source. Significant observations are as follows:

a. Furan-1 was blistered on steel in the water phase in the inoculated tests. No blistering in the controls was evident.

b. Metal-1 has failed by loss of adherence of the topcoat.

c. Buna-N type coatings show the usual leaching of color in the fuel phase and bleaching in the water phase. Blistering appears incipient.

6. Disc Assay Tests

In general, the disc assay tests confirm the nitrogen and carbon-free media experiments, since the general pattern is one of growth stimulation. Stimulation on these tests is variable and difficult to estimate. Frequently one finds slight inhibition and a subsequent zone of growth stimulation presumably due to the Colley effect, i.e., growth stimulation from low concentrations of toxicants.

There are three major exceptions to the general effect of stimulation. Chromate polymerized polysulfide coatings are toxic as are the water extracts from Furan 1 and Buna-2. These data are supported by the data concerning the use of the coatings as a nitrogen source where less growth was obtained with the extracted coating. Presumably in the case of the last two coatings the toxic material is largely removed by water extraction. Water extraction does not seem to remove all of the chromate from the polysulfide, and residual toxicity is present.

7. Coupon Tests

The data on the coated coupons provide two coating failures. The Metal-1 failure is presumably due to lack of adhesion of the topcoat to the primer.

The Furan 1 coating is failing by blistering in the water phase. So far, the data show this to be a microbiological failure. Blistering has occurred only where growth was present and not in the controls. An accidential internal confirmation is noted in one flask that was inoculated but in which growth did not occur; there is no blistering on this coupon. This series provides the first clear-cut visual coating failure connected with microbial growth.

TABLE 17

VISUAL OBSERVATIONS OF COATED COUPONS

Coating	Substrate and Color	Color Extraction	Growth	Comment
Polysulfide -1	Aluminum gray	None	good(c)	Coating slightly faded in water
Polysulfide -1	Steel gray	None	good(c)	Slightly faded in water layer
Buna -1	Aluminum red	Red in fuel	(c)	Dye leaches out leaving 3 distinct bands of color
Buna -1	Aluminum red	Red in fuel	heavy	Dye leaches out leaving 3 distinct bands of color
Buna -1	Aluminum red	Red in fuel	heavy	Dye leaches out leaving 3 distinct bands of color
Buna -1	Aluminum red	Red in fuel	heavy	Dye leaches out leaving 3 distinct bands of color
Buna -1	Steel red	Red in fuel	good	Dye Mottled at fuel phase. Incipient blistering
Polyurethane -2	Steel yellow-green	None	none(c)	No effect
Polyurethane -2	Steel yellow-green	None	good	No effect
Polyurethane -2	Steel yellow-green	None	good	No effect
Polyurethane -2	Aluminum yellow-green	None	none(c)	No effect
Polyurethane -2	Aluminum yellow-green	None	Light	No effect
Furan -1	Aluminum black	None	none(c)	Swelling in water layer
Furan -1	Aluminum black	None	heavy	Swelling in water layer
Furan -1	Steel black	None	good(c)	Blistered in water phase

TABLE 17 (Continued)

Coating	Substrate and Color	Color Extraction	Growth	Comment
Furan -1	Steel black	None	Good	Blistered in water phase
Furan -1	Steel black	None	heavy	Blistered in water phase
Buna-4	Steel red	in fuel	Light	Dye leaches in fuel. Coating formed bubbles when applied
Buna-4	Aluminum red	in fuel	None	Dye leaches in fuel. Coating formed bubbles when applied
Buna-4	Aluminum red	in fuel	heavy	Dye leaches in fuel. Coating formed bubbles when applied
Buna-3	Steel red	in fuel	moderate	Dye Slight Mottling
Buna-3	Aluminum red	in fuel	heavy	Dye Slight Mottling
Metal-1	Steel red	None	(c)	Peeling of topcoat
Metal-1	Steel red	None	good	Peeling of topcoat
Metal-1	Aluminum red	None	heavy	Peeling of topcoat
Polyurethane -1	Steel white	None	slight	No effect
Polyurethane -1	Aluminum white	None	None	No effect
Polyurethane -1	Aluminum white	None	heavy	No effect
Buna-2	Steel red	In fuel	(c)	Color fading, coating bubbled when applied. Blistering appears to be occurring in water phase
Buna-2	Steel red	In fuel	slight	Color fading, coating bubbled when applied. Blistering appears to be occurring in water phase

TABLE 17 (Continued)

Coating	Substrate and Color	Color Extraction	Growth	Comment
Buna-2	Aluminum red	in fuel	(c)	Color fading, coating bubbled when applied. Blistering appears to be occurring in water phase
Buna-2	Aluminum red	in fuel	heavy	Color fading, coating bubbled when applied. Blistering appears to be occurring in water phase
Polysulfide-2	None brown	none	(c)	Color fading
Polysulfide-2	None brown	none	heavy	Color fading

NOTES:

1. (c) indicates a control. Growth is occasionally recorded as the result of unsterilized coupons.

2. Polysulfide -2 is tested as a fillet; i.e., there is no substrate.

3. Buna-2 is probably in the first stage of blistering. It is difficult to distinguish blistering from the bubbling that occurred when the panel was coated.

4. Coupons are incubated under static conditions at 28°C in TS-11 plus JP-4.

8. Resistance Tests

As mentioned previously the tests on the electrical resistance of the coatings on aluminum and steel should be regarded as very rough approximations. Their purpose was to determine whether differences could be detected in resistance that would warrant further an exploration of the technique. The preliminary data indicate that resistance measurements may provide at least an adjunctive tool to visual observations. This is confirmed by the literature on coating deterioration. (Ref. 5).

V. REFERENCES

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